



# EDGEWOOD

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U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

ECBC-TR-510

**VAPOROUS HYDROGEN PEROXIDE (VHP) DECONTAMINATION  
OF A C-141B STARLIFTER AIRCRAFT:  
VALIDATION OF VHP AND MODIFIED VHP (mVHP)  
FUMIGATION DECONTAMINATION PROCESS  
VIA VHP-SENSOR, BIOLOGICAL INDICATOR, AND  
HD SIMULANT IN A LARGE-SCALE ENVIRONMENT**

Mark D. Brickhouse  
Abe Turetsky  
Brian K. MacIver  
Jerry W. Pfarr  
Theresa A. Lalain

RESEARCH AND TECHNOLOGY DIRECTORATE

Iain McVey  
Wendlyn Alter  
STERIS CORPORATION  
Mentor, OH 44060

John P. Lloyd  
Mark A. Fonti, Jr.  
20<sup>th</sup> SUPPORT COMMAND (CBRNE)

STERIS<sup>®</sup>



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## 1. INTRODUCTION

The C-141B Starlifter aircraft vaporous hydrogen peroxide/modified vaporous hydrogen peroxide (VHP/mVHP) decontamination tests are part of a congressionally funded joint venture between U.S. Army Edgewood Chemical Biological Center (ECBC) and Strategic Technology Enterprises, Inc. (STE), a subsidiary of STERIS Corporation, Inc. (Mentor, OH). The tests were performed between Oct and Nov 2004 in a remote area within the Air Maintenance and Reclamation Command (AMARC), Davis-Monthan Air Force Base, Tucson, Arizona.

The primary objective of these tests was to determine the effectiveness of the mobile VHP-CB (mVHP™) system developed by STERIS in distributing the VHP/mVHP fumigant evenly throughout the aircraft for the purpose of decontaminating substrates contaminated with chemical and biological warfare (CBW) threat materials while maintaining a near constant 250-ppm fumigant concentration and varying duration of exposure. Biological indicators (BIs) and a variety aircraft related materials, in the form of coupons, were contaminated with biological and chemical challenges. The test materials were strategically placed throughout the aircraft (cargo area volume: 13,000 cu. ft). The coupons were exposed to the fumigant at the target concentration level during three VHP runs of different time periods (5-, 10.5- and 24-hr) and one 24-hr mVHP run. Over 99.5% kill was achieved for the BIs and in two of the VHP runs the residual CBW levels were below the threshold levels set by the Joint Portable Interior Decontamination System (JPIDS) Operational Requirements Document (ORD).

The VHP/mVHP technology was also examined to determine fumigant compatibility with sensitive materials. The structural components of the aircraft and the coupons were carefully checked for any signs of decomposition from exposure to the fumigant. No material degradation was found. The resulting data has conclusively established that the VHP/mVHP technology is effective in decontaminating an aircraft interior without degrading any structural components.

### 1.1 Background

The possibility of the release of CBW agents and toxins has generated the need for fast, effective and environmentally safe methods of decontamination. The Department of Defense (DoD) is interested in developing a decontamination technology for military relevant surfaces. Other vaporous decontamination technologies include toxic gases such as formaldehyde and ethylene oxide. Though these two gases are effective decontaminants, they are carcinogenic and potentially explosive besides being highly toxic. The VHP ( $H_2O_2$ ) fumigant appears to be the safest vaporous decontaminant in existence to date. It requires no neutralization prior to release due to its rapid decomposition into two environmentally benign products: oxygen and water vapor (Figure 1).



The VHP<sup>®</sup> technology developed by STERIS (EPA registration #58779-4) has been in use for more than a decade. The VHP fumigant was initially used to sterilize pharmaceutical processing equipment and clean rooms.<sup>1,2</sup> In Oct 2001, the VHP technology was adapted to decontaminate two anthrax-contaminated buildings in the Washington, D.C. area. The VHP system used in the anthrax remediation has been modified and is now more modular and easier to transport. The new system (mVHP<sup>™</sup>), used in the C-141B aircraft decontamination tests, has been successfully demonstrated in building tests at the Aberdeen Proving Ground (APG), MD.<sup>3</sup>

In addition to its biological efficacy, the VHP technology has been modified to include the decontamination of materials contaminated with toxins and chemical agents such as VX and HD. During the chemical efficacy testing of the VHP fumigant against VX, GD, and HD in early decontamination studies conducted by ECBC, GD was observed to be quite stable in the presence of VHP. However, the addition of a low level of ammonia gas (NH<sub>3</sub>) was found to render VHP reactive to GD. Thus VHP activated with ammonia gas, mVHP, has been proven to permit broad-spectrum decontamination of VX, GD, and HD.<sup>4</sup>

The mVHP technology has been developed and patented through an initial Cooperative Research and Development Agreement (CRADA) between ECBC and STE, a Subsidiary of STERIS Corporation, Inc.

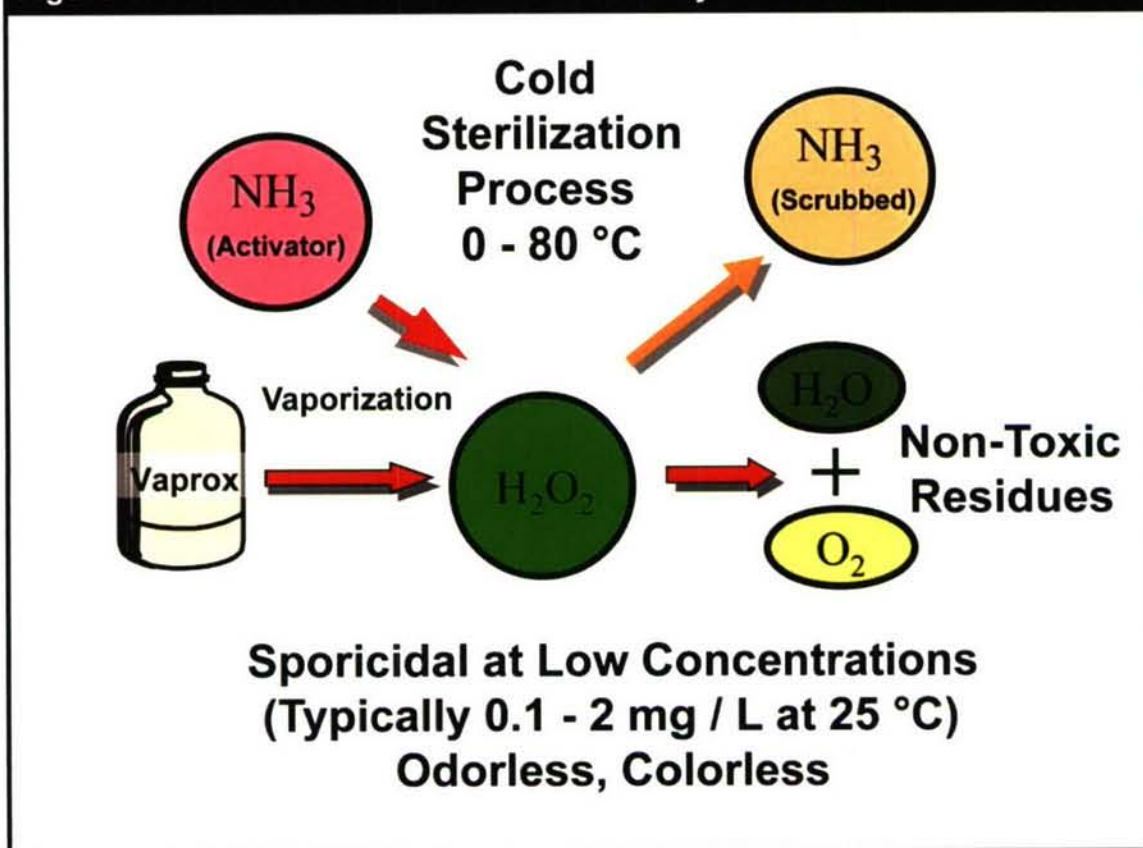
## 1.2 The mVHP<sup>®</sup> Decontamination Process

When released in vaporized form, hydrogen peroxide forms hydroxyl free radicals that react with various micromolecules such as proteins, lipids, RNA and DNA. Vaporized hydrogen peroxide (VHP) also reacts with and neutralizes VX and HD chemical agents.<sup>4</sup> When activated by small amounts of ammonia (approximately 15 ppm by volume), VHP becomes reactive with GD as well, offering broad spectrum decontamination of chemical- and biological-agents. VHP modified with ammonia is referred to as mVHP.

The mVHP decontamination process is effective at atmospheric pressure within a broad range of ambient temperatures. Unreacted hydrogen peroxide readily decomposes to form water and oxygen, leaving no toxic residues (Figure 1). The ammonia concentration used is well below the Permissible Exposure Limit (PEL) of 50 ppm and is scrubbed out of the exhaust air through an appropriate filter.

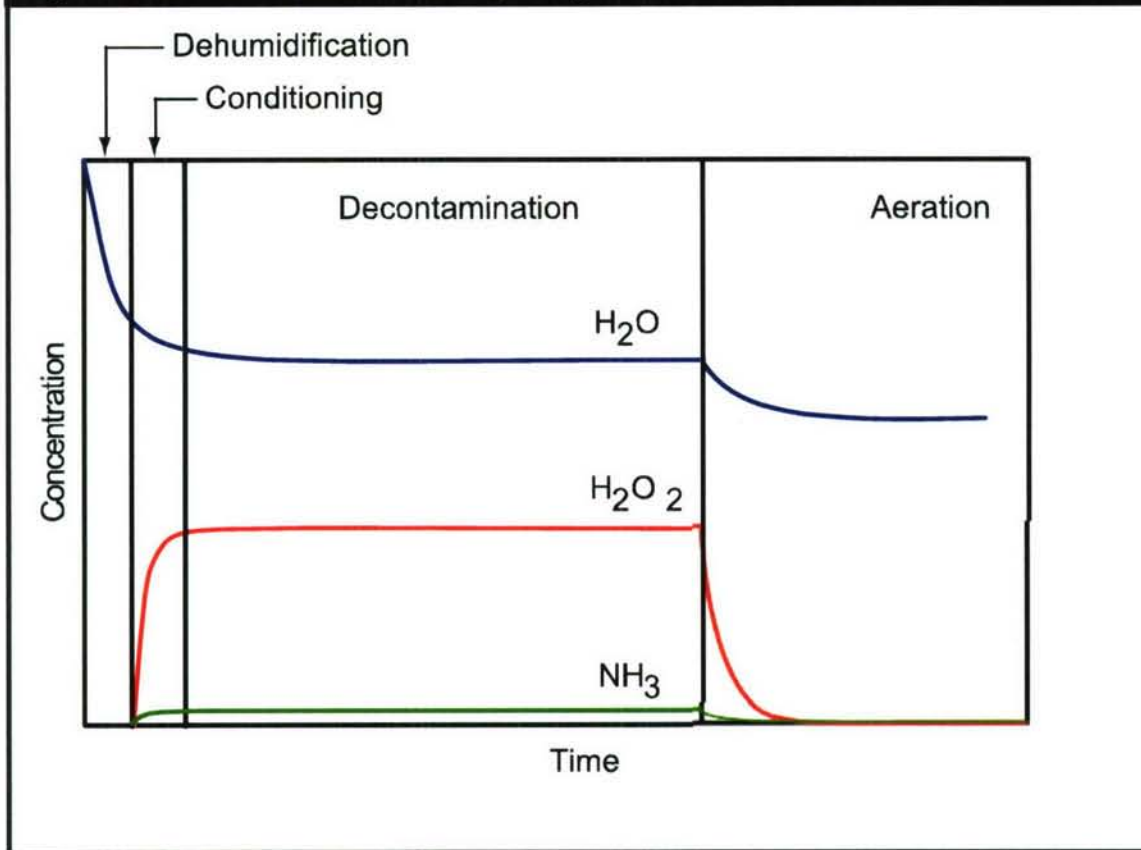


**Figure 1: mVHP<sup>®</sup> Decontamination Chemistry**



Decontamination of an interior space using the modular mVHP system is a four-phase process involving preparation of the interior air (dehumidification), achieving a steady state decontaminant level (conditioning), performing the decontamination, and then aerating the interior space for safe entry (Figure 2).

**Figure 2: mVHP® Decontamination Cycle**



#### Dehumidification

Hydrogen peroxide vapor can co-condense with water vapor producing an undesired condensate high in hydrogen peroxide. If ambient conditions are likely to permit condensation – high humidity and/or cold temperatures – potential condensation can be prevented by circulating dry, heated air through the interior space prior to injection of the hydrogen peroxide vapor. The target humidity level is determined by the concentration of vapor to be injected and the desired steady state concentration for the decontamination. The lower relative humidity permits a higher hydrogen peroxide concentration without reaching a saturation point.

#### Conditioning

During the conditioning phase, injection of ammonia and hydrogen peroxide vapor is initiated. Injection rates are selected to rapidly raise the concentrations to the desired set point without condensation. Internal sensors measure and report the ammonia and hydrogen peroxide concentrations to the control system. When the set-point concentrations values are reached, the ammonia and hydrogen peroxide injection rates are lowered to maintain the set-point concentrations. Once all the interior monitors reach or exceed the set point concentration, the system proceeds to the next phase.

## Decontamination

Decontamination is a timed phase dependent on the hydrogen peroxide vapor concentration, ammonia vapor concentration and temperature. A decontamination timer counts down from the preset decontamination time. The timer stops if the concentration or temperature values fall below the set-point. The timer ensures that during the decontamination phase, the interior space is exposed to at least the minimum decontamination conditions for the desired exposure time.

## Aeration

After completion of the decontamination phase, the system stops injection of hydrogen peroxide and ammonia and introduces only dried air into the interior space. The dried air displaces the hydrogen peroxide and ammonia. The hydrogen peroxide and ammonia are removed by the exhaust system. Samples are drawn and tested from the exhaust system upstream of the catalyst destroyer. When the measurements are below the ammonia and hydrogen peroxide PELs, the user terminates the aeration process.

### 1.3 Modular mVHP System

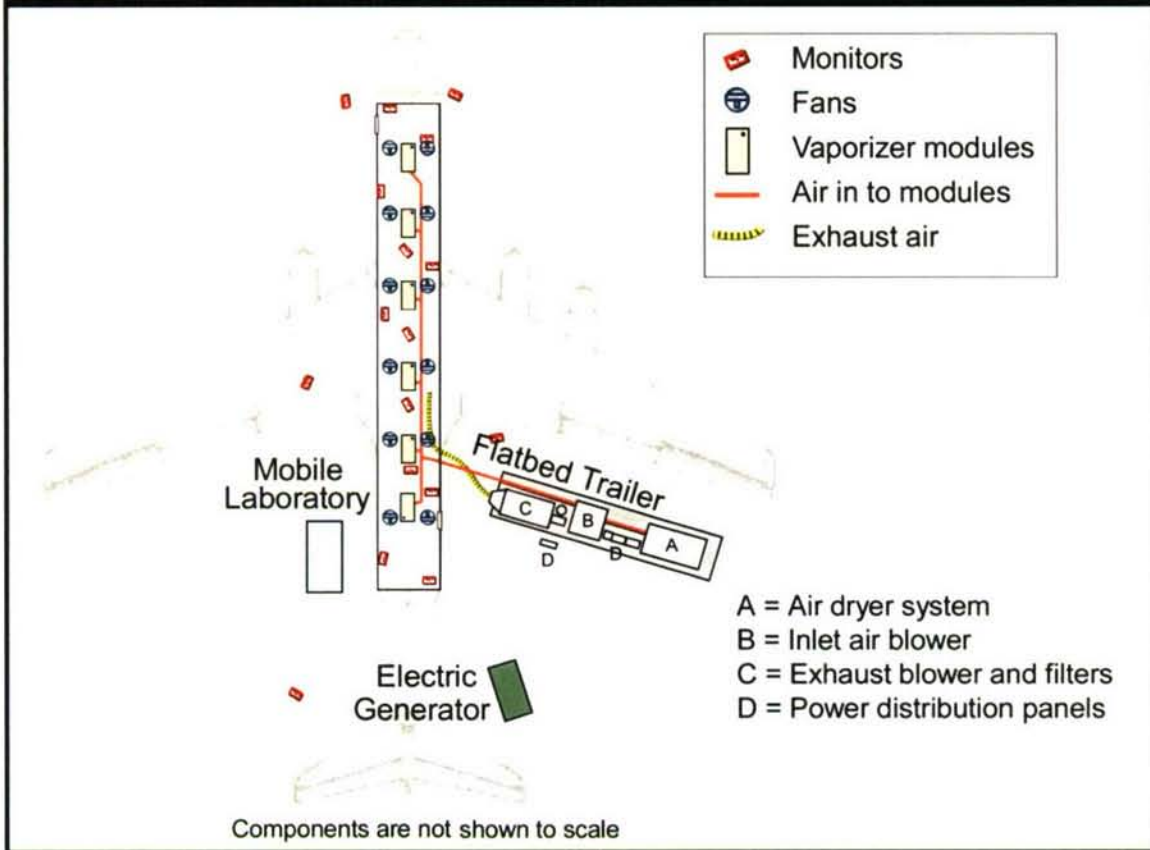
During the GSA and Department of State anthrax decontaminations, STERIS employed a system design that placed vaporizer units exterior to the building along with the air handling system and the exhaust system. The buildings were divided into sealed zones that were sized to the capacity of the decontamination system, and VHP vapor was injected through ducting into each zone in turn. The original units were limited to a fixed interior size and configuration.

A modular system was created capable of rapidly accommodating a wide variety of interior sizes and configurations. The modular design was successfully utilized in the building demonstration.<sup>3</sup> The modular design was adopted for the current demonstration (Figure 3). The approach involves placing an appropriate number of vaporizer modules, associated monitors and distribution fans inside the area to be decontaminated. Air handling, exhaust and control systems are located exterior to the space. Vapor concentrations, temperature and relative humidity monitor measurements are reported to a single centralized control system.

The modular mVHP system was used to deliver, distribute and monitor both the VHP and mVHP fumigants to the interior of the C-141B aircraft. The major mVHP system components and their orientation relative to the aircraft are presented in Figure 3 and are discussed in detail in Section 2.2. The decontamination results discussed in Section 3 demonstrate that the modular mVHP system can successfully decontaminate complex spaces such as a C-141B Starlifter aircraft cargo interior.



**Figure 3: Modular mVHP® System Components for C-141B Demonstration**



The modular mVHP system components include:

- Air handling system (air dryer and blower) – provides dehumidified process air to the vaporizer modules, ensuring that mVHP condensation will not occur.
- Ducting – delivers process air from the air handling system to each of the vaporizer modules, and exhausts interior air to the negative air system.
- Vaporizer modules – heat the process air, generate mVHP from hydrogen peroxide and ammonia solutions, control the fans, and provide process control and monitor data to and from the central control system.
- Vapor distribution fans – distribute mVHP throughout the interior space.
- Exhaust/Negative air system – draws air out of the interior space at a slightly higher flow rate than it enters, to prevent breach of containment. The air is filtered through a HEPA filter, catalyst bed and carbon filter before release into the environment.



- Control system – provides a single point of control for all modules and system components. The user interface enables the system operator to view sensor readings, set or modify process parameters, and monitor trends.
- Internal monitors – continuously monitor hydrogen peroxide concentration, ammonia concentration, temperature, and relative humidity throughout the interior space and provide real-time feedback to the central control system.
- External monitors – stand ready to trigger an alarm at the central control system, notifying the operator if hydrogen peroxide or ammonia levels above Permissible Exposure Limits (PEL) are detected outside the containment zone perimeter (i.e., containment failures are detected).

## 2. METHODS AND PROCEDURES

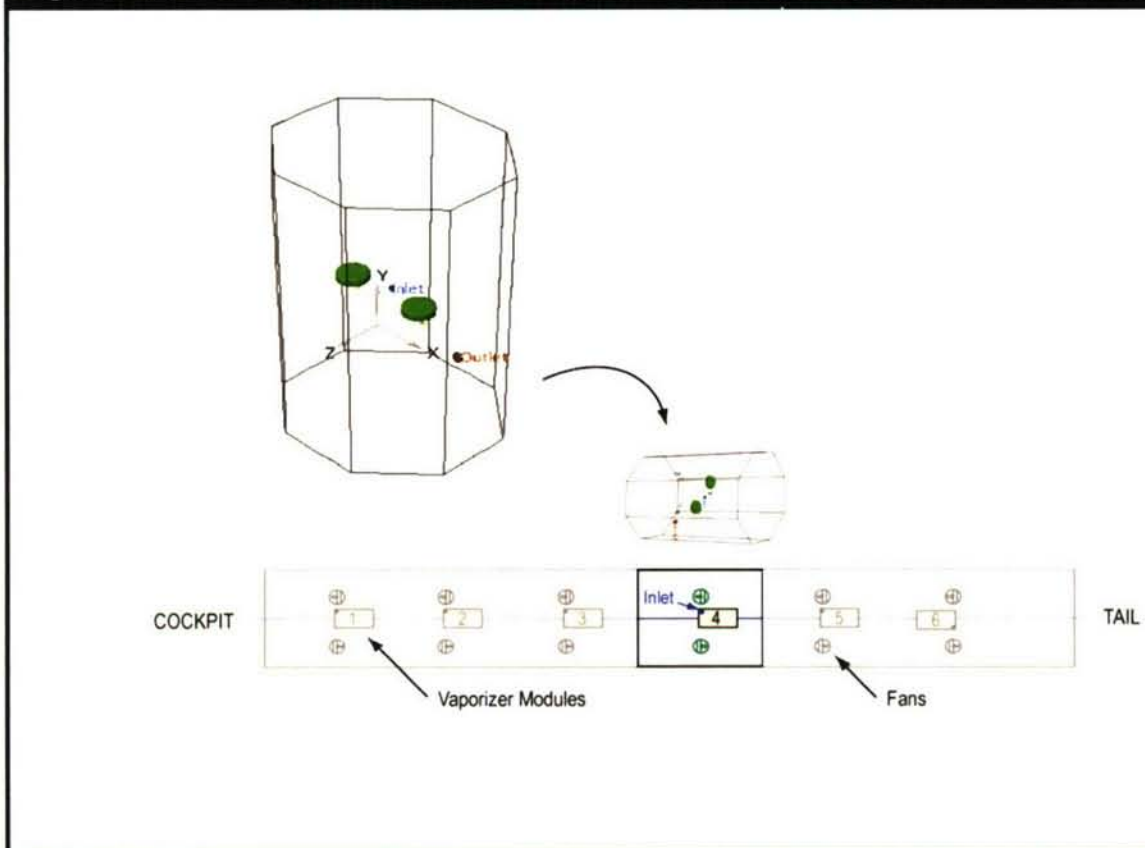
### 2.1 Computational Flow Dynamics (CFD)

In order to determine the placement of the fans and vaporizer modules that would optimize vapor distribution throughout the cargo hold, a CFD model was developed. CFD obtains numerical solutions to fluid flow problems by using a set of equations that govern the motion of fluids. These include the continuity (conservation of mass), the Navier-Stokes (conservation of momentum), and the energy equations, which form a system of second order, non-linear partial differential equations. The differential equations are reduced to a set of algebraic equations, which can then be solved with the aid of a computer to get an approximate solution to fluid flow. CFX CFD software (ANSYS, Inc.), which employs an enterprise accessible software applications web-based front end, was used in modeling the airflow.

To simplify the computational demands of the model, the cargo hold was considered to be an extruded octagon equivalent to the cylindrical shape of the cargo area (diameter = 163 in., length = 1258 in.). The model assumed that six vaporizer modules would be placed along the length of the hold; each module would control two fans; and the airflow around each module would be similar. A segment of the hold, corresponding to 186 in. and containing one vaporizer module, was modeled (Figure 4). For ease of modeling, the segment was oriented with the axis of the aircraft on the vertical Y axis.

The vapor nozzle, inlet, of the vaporizer module was positioned near the center of the cross-sectional diameter at a location on the Z axis corresponding to 54 inches. The experience gained in the building decontamination demonstrations at APG suggested that the best distribution would be obtained by creating a circular flow pattern. A fan was positioned on each side of the vaporizer nozzle (X axis). The two fans associated with each module were positioned to move air in opposite directions to promote uniform fumigant concentration. The model did not account for gravitational effects (-Z axis) or the anti-gravitational orientation of the nozzle (+Z axis).

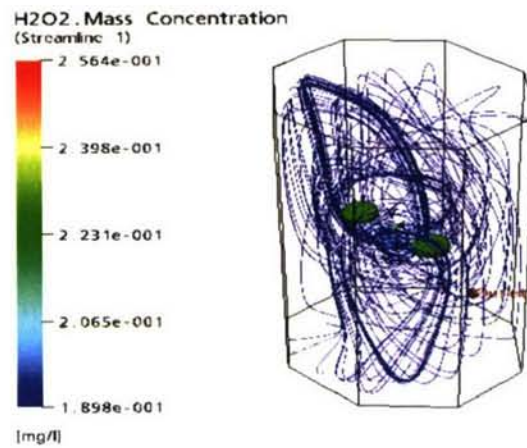
**Figure 4: Inlet, Outlet and Fan Placement within One Cargo Area Segment**



Based on experience gained from the building demonstrations, the model injection rate was set at 3 g/min  $\text{H}_2\text{O}_2$  with airflow of 133 cfm. The temperature of air within the enclosure was assumed to be 25 °C and the temperature of the inlet air was set at 95 °C (> 90 °C is required for vaporization).

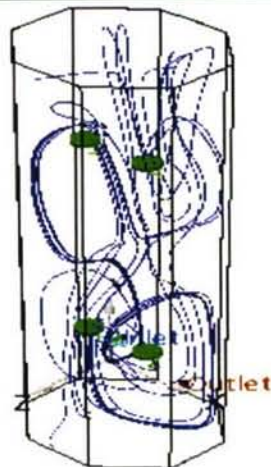
Although the model considered the cross-sectional ends of the segment to be closed, resulting streamlines were expected to be representational, as modeling conducted with two sets of fans showed that each pair set up its own convection cell, along with some cross-flow (Figure 5).

**Figure 5: Streamline Concentration Distribution (Top View)**



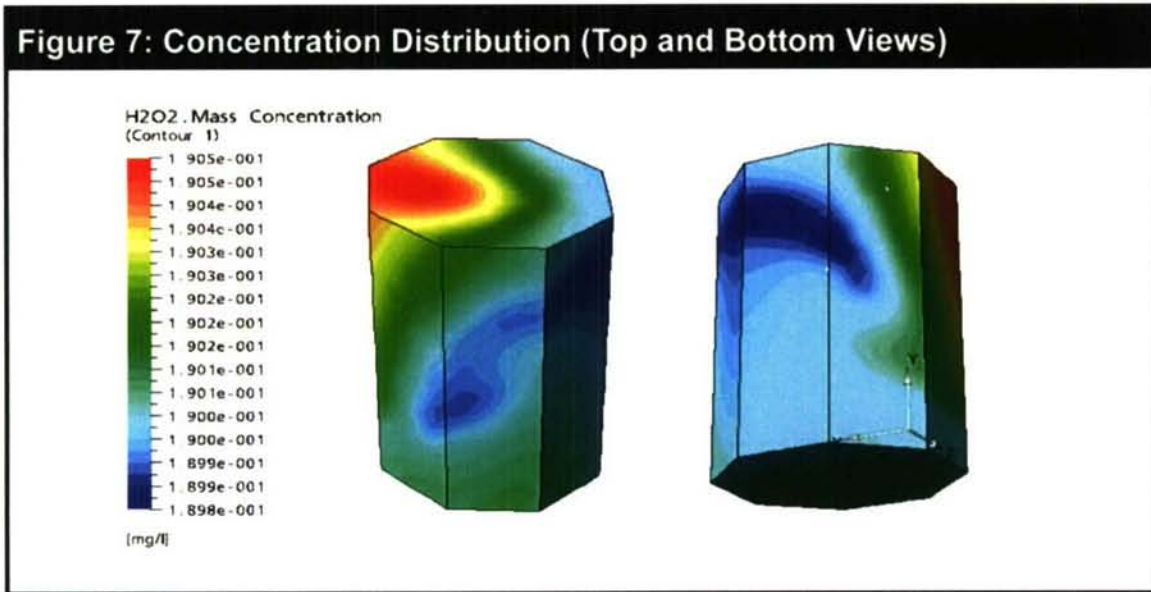
For the single inlet, two fan configuration, predicted VHP concentrations at the walls of the cylinder, cargo hold surfaces, are indicated by concentration distribution plots (Figure 6).

**Figure 6: Convection Cells for Two Sets of Fans**





The difference between the maximum and minimum mass concentration presented in Figure 7 (1.905e-001 and 1.898e-001) illustrates that for this configuration and fan placement, the CFD model predicted complete mixing to within less than half a percent.



The final orientation of fans included a slight upward tilt rather than the horizontal orientation used in the flow dynamics model. In the building decontamination tests, this was found to provide improved distribution to the upper corners of the rooms.

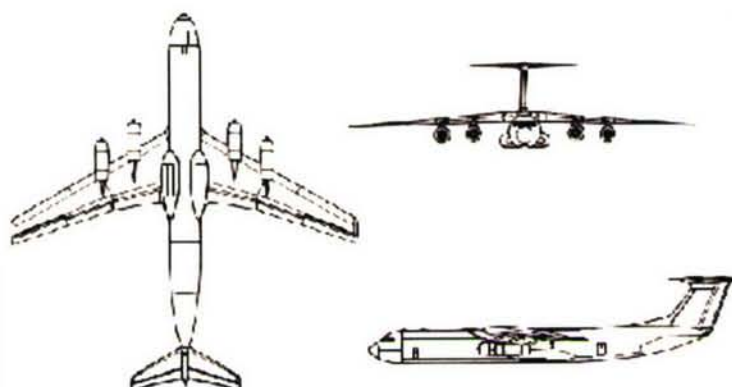
## 2.2 Modular mVHP® Aircraft Site Preparation and Components

The C-141B Starlifter, designed for long-range troop and cargo airlift, has a cargo length of 168 ft 4 in., a height of 39 ft 3 in., and a wingspan of 160 ft (Figure 8). The cargo load capacity is rated at 6,370 cu ft but for decontamination purposes, the total cargo area volume was taken as approximately 13,000 cu ft of air. A photograph of the cargo area volume is shown in Figure 8b.



**Figure 8: C-141B Aircraft Cargo Area**

a) Schematic and Dimensions



Inches	Cargo Area
1258	Length from forward cockpit wall to aft pressure door
122	Width at floor
163	Width at widest point

b) Photograph



### 2.2.1 Site Organization and Preparation

A crew of six engineers and technicians was assigned to deploy and operate the system during the decontamination demonstration. Initial planning included a reconnaissance trip to determine onsite requirements. The modular mVHP system and ancillary equipment were transported to the site in two enclosed tractor-trailers and one flatbed trailer. The actual setup of the mVHP system and ancillary equipment took place during Oct. 19 and 20. To minimize setup operations, the air handling and exhaust systems were operated directly from the flatbed trailer in their transport configuration.

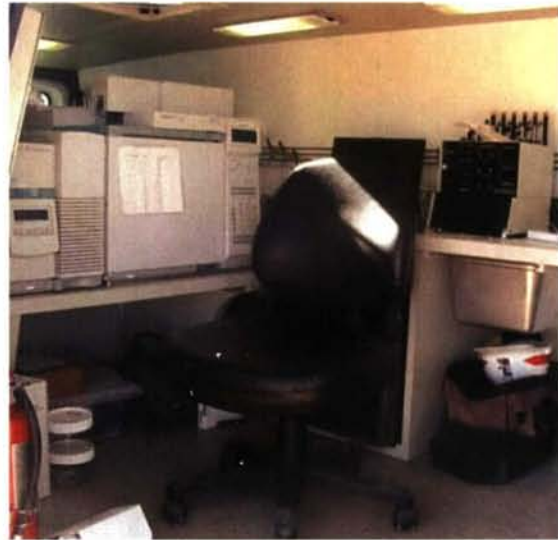
The decontamination demonstration was entirely self-contained. A 230-V, 3-phase, 250-kW portable electric generator and an office trailer, for housing the control system, were rented locally. ECBC provided two vans equipped as mobile laboratories. One van was equipped with incubators, a laminar flow hood, and an automated colony counter for use as a microbiological laboratory (Figure 9a). The second was equipped with a gas chromatograph and was used for the analysis of chemical coupons (Figure 9b). Both, the office trailer and the mobile chemical analysis laboratory, operated on power drawn from the generator. AMARC provided floodlights for night operations, two portable stair units, a port-a-potty, and a “bowser” of non-potable water.

**Figure 9: Mobile Laboratories**

a) Microbiology Van Interior



b) Chemical Analysis Van Interior



### 2.2.2 Air Handling System

The air handling system consists of an air dryer and a blower (Figure 10). The air dryer, a Trane air conditioning unit and a Munters industrial desiccant dehumidifier, removed the majority of the water vapor from the incoming air throughout the decontamination process. The Sonic Air System 350 blower provided forced air circulation. An anemometer, located downstream of the main blower, measured and recorded the airflow generated by the mVHP system. The location of the blower is depicted in Figure 12.

**Figure 10: Air Handling System on Flatbed Trailer**



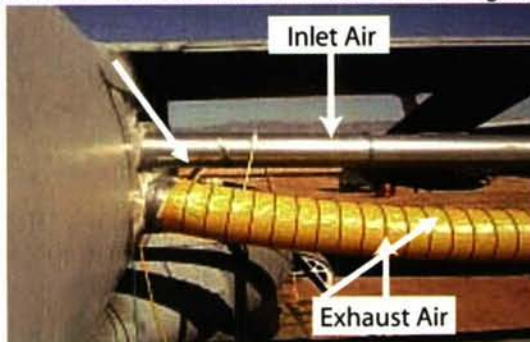


### 2.2.3 Ducting

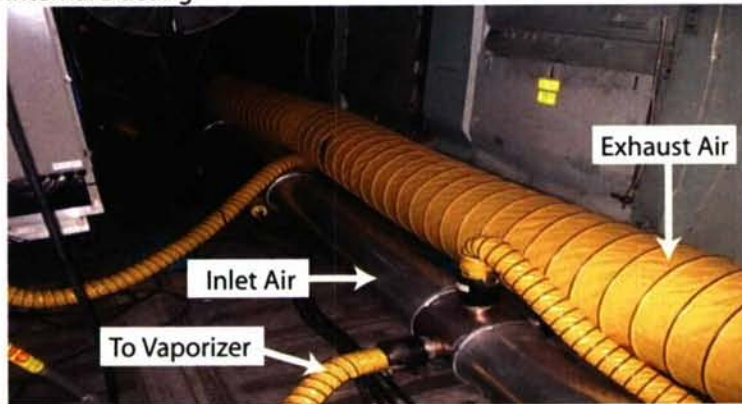
Modular rigid and flexible ducting provided the capability to tailor the air handling system to a wide variety of interior configurations (Figure 11a). Dehumidified air was delivered from the air handling system into the aircraft through a galvanized steel duct. As illustrated in Figure 11b, flexible ducting inside the aircraft was used for both the exhaust system and for delivering conditioned air to each vaporizer module.

**Figure 11: Ducting**

**a) Air Inlet and Exhaust Manifold Pass-Throughs**



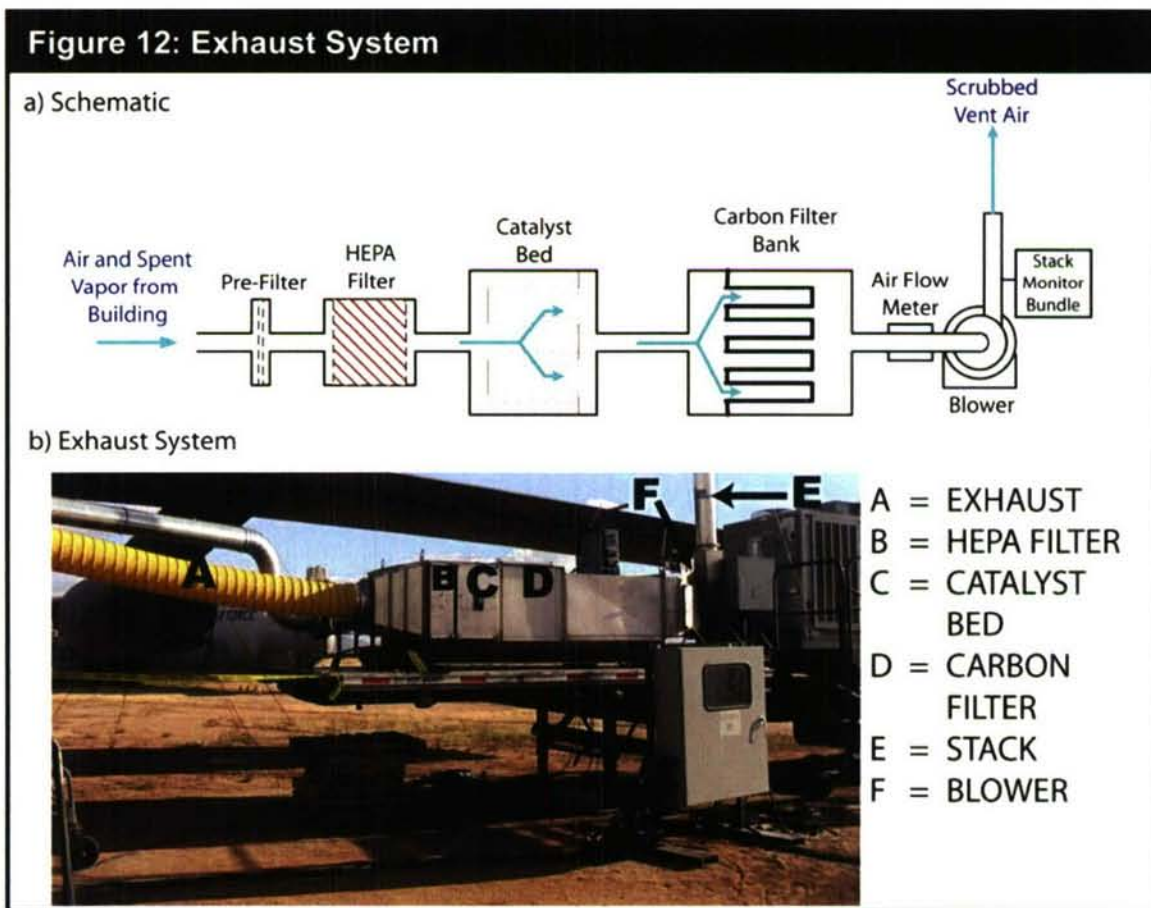
**b) Internal Ducting**



## 2.2.4 Exhaust/Negative Air System

Spent vapor was exhausted from a central location within the aircraft through the flexible ducting. The blower attached to the exhaust unit drew air from the aircraft at a slightly higher flow rate than the air entering the aircraft via the air handling unit. Maintaining negative pressure inside the aircraft ensured the containment of the VHP/mVHP fumigant, even when the aircraft was not fully sealed (during the 24-hr VHP run, air flowed into the aircraft through leaks at the tail).

Spent vapor passed through a high efficiency particulate air filter for microbial retention prior to exhausting the air. Any remaining hydrogen peroxide was reduced to water and oxygen during passage through a palladium/platinum catalyst bed, and a carbon filter removed ammonia from the exhaust stream before it was vented through the stack (Figure 12). A control system warning alarm was set to trigger if hydrogen peroxide, ammonia, or the chemical agent simulant were detected at levels above the permissible exposure limits (PEL) in the stack.





### 2.2.5 Vaporizer Modules

The vaporizer modules used for the aircraft decontamination process have a capacity to decontaminate approximately 5000 cu. ft. Each is equipped with two pressurized bottles of ammonia and two Vaprox<sup>®</sup> (35% hydrogen peroxide) carboys. During the runs, the carboys were continuously weighed to monitor H<sub>2</sub>O<sub>2</sub> usage. The interior temperature of the aircraft was controlled by raising the temperature of the air from the air handling system with the use of three independently controlled pre-heaters.

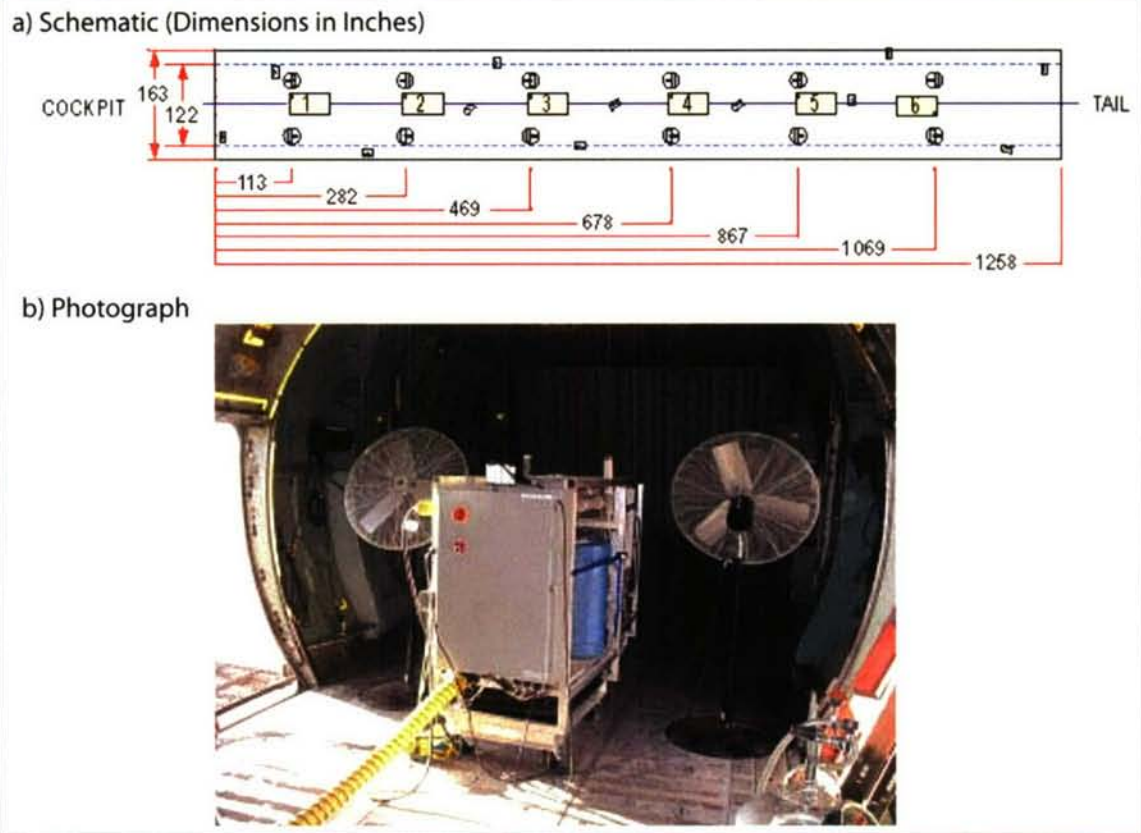
Six vaporizer modules were positioned along the length of the cargo bay, approximately centered along the longitudinal axis (Figure 13a). When the 24-hr VHP run showed low vapor concentration at the tail of the aircraft due to air leakage around the aft cargo door, the problem was successfully corrected by moving units five and six and boosting the unit injection rates. For the 5- and 10.5-hr VHP runs and the 24-hr mVHP run, the vaporizer locations were as follows: Vaporizer 5 was taken offline, monitors 5A and 5B were attached to Vaporizer 6, and monitors 6A and 6B were attached to Vaporizer 7. The new designations in the tables are Vaporizers 6\* and 7\*. The new configuration relocated the two vaporizers toward the rear of the aircraft to counteract the dilution of the incoming air. Moving a vaporizer unit further aft than 1069 in. was not possible because the loading ramp sloped at a sharp angle (Figure 16).

A floor fan was placed on either side of each vaporizer module (Figure 13b) and two monitors were positioned nearby. Each vaporizer module was fitted with a control module, which conveyed data to the central control unit from the vaporizer module and the two monitors via a 4-20-mA signal cable. The control module also received control signals for the two floor fans and each vaporizer module valves and heaters.

### 2.2.6 Vapor Distribution Fans

Each vaporizer module controlled two high capacity (~7000 cfm) floor fans that distributed the mVHP vapor throughout the interior. The fans were a special build (230-V motors were employed to provide compatibility with the power distribution systems of the modules) manufactured by Marley Engineered Products.

**Figure 13: Vaporizer Module and Fan Placement**



### 2.2.7 Internal Monitors

Internal and external monitors provided continuous feedback to the control system regarding the measured temperature,  $\text{H}_2\text{O}_2$  concentration (ppm),  $\text{NH}_3$  concentration (ppm), and relative humidity. Tracking vapor concentration and water saturation prevented conditions where condensation could occur, and the VHP/mVHP injection rate was accordingly adjusted. This ensured that vapor concentration was steadily maintained at the target concentration level throughout the duration of a decontamination run.

The water vapor sensors, manufactured by Vaisala, were packed with manganese oxide ( $\text{MnO}_2$ ) catalyst to protect the sensing element from cross sensitivity to the hydrogen peroxide vapor. The temperature within the aircraft was measured using stainless steel-sheathed platinum resistance temperature devices.

Twelve internal monitors were placed at different heights throughout the aircraft to monitor process parameters and provide feedback to the control system (Figure 14). Fan and monitor configuration in relation to each vaporizer module is depicted in Figure 14. Table 1 presents the approximate position of each monitor within the aircraft.

<b>Table 1: Monitor Postitions in the C-141B Aircraft</b>			
<b>Monitor</b>	<b>Height (Inches)</b>	<b>Y* (Inches)</b>	<b>X* (Inches)</b>
1A	66	14	11
1B	69	110	89
2A	52	-11	225
2B	16	55	378
3A	15	0	542
3B	67	124	418
4A	55	61	594
4B	55	60	778
5A	80	67	947
5B	35	137	1001
6A**	63	-5	1176
6B**	31	114	1233

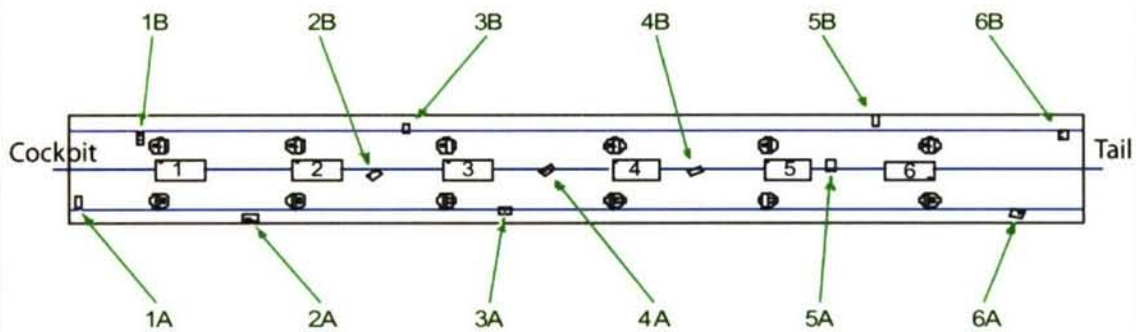
\* Measured from point where floor meets wall at fore bulkhead

\*\*These measurements are based on the actual elevation above the floor line, not the height above the angled tailgate floor

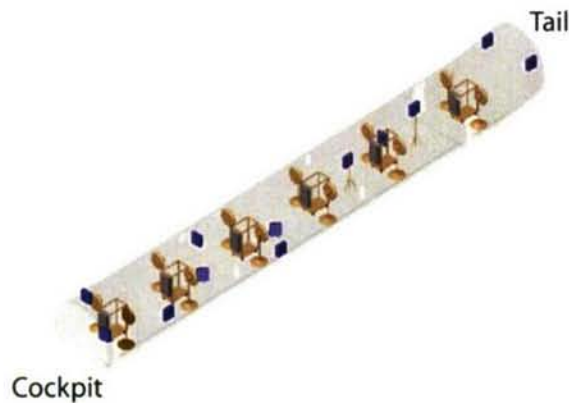


**Figure 14: Internal Monitor Placement**

a) Schematic



b) Schematic - 3D CAD Drawing



c) Photographs

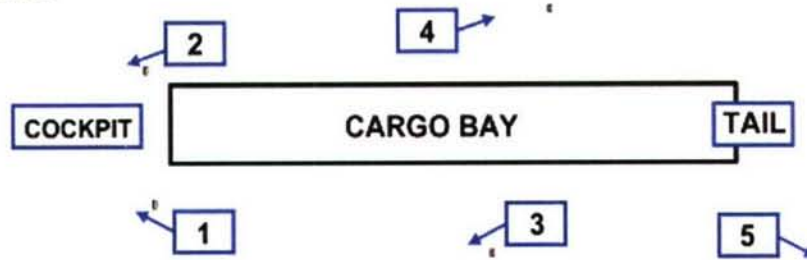


### 2.2.8 External Monitors

Five external monitors were placed around the perimeter of the aircraft to ensure that decontaminant levels remained below PEL (Figure 15a). Figure 15b depicts monitor one positioned near the cockpit of the aircraft. Monitors three and five can be seen in the distance. ATI and A12 sensors were used for tracking both VHP and ammonia during each phase of the decontamination process. Alarms were set to trigger at the PEL for either component of the VHP/mVHP fumigant: 50 PPM for  $\text{NH}_3$ , 1 ppm for  $\text{H}_2\text{O}_2$

**Figure 15: External Monitor Placements**

a) Schematic



b) Photograph, Showing Monitor 1 and Monitors 3 and 5 in distance



### 2.2.9 Electrical Generator

Power for the mVHP system was supplied by a 230-V, 3-phase, 250-kW mobile electrical generator located external to the aircraft (Figure 16a). All connections to the generator were weatherproof. A power distribution module supplied power to the air handling system and exhaust unit (Figure 16b). A second power distribution module routed power from the generator to the vaporizer modules inside the aircraft (Figure 16c). Circuit breakers on both power distribution modules were rated for each component. The generator also provided power to the office trailer, the mobile chemical laboratory, and the floodlights.

**Figure 16: Electrical Generator and Power Distribution Panels**

a) Electrical Generator



b) Power Distribution Panel to Vaporizer Modules



c) Power Distribution Panel to Air System and Exhaust



#### 2.2.10 Integrated Control System

The control system (Figure 17a,b) was located adjacent to the aircraft in a climate controlled rented office trailer (Figure 17c). All the components of the control system operated under a single controller. The control system monitor displayed and recorded all monitored process variables, which enabled real-time adjustment during the process and the detailed analysis conducted following the process.

Decontamination process variables monitored by the control system include

- Air flow rates through the air handling and exhaust units;
- Hydrogen peroxide and ammonia concentrations;
- Relative humidity readings accumulated at the aircraft interior monitors; and
- Temperature of the vaporizer module heaters.

Although the decontamination process was automated, the system operator could monitor and adjust conditions within different areas of the aircraft interior; activate or deactivate individual components; and adjust settings at any point during the process.

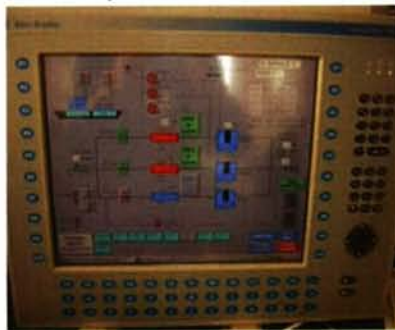


**Figure 17: Integrated Control System**

a) Control System Operator Station



b) Control System



c) Control System Housed in Adjacent Trailer



### 3. DECONTAMINATION DEMONSTRATION: TESTING AND ANALYSIS

#### 3.1 Preparation of Aircraft for Decontamination

STE personnel prepared the aircraft for decontamination. Placement of vaporizer sensor bundles and fans and the installation of the ductwork and exhaust system were carried out as presented in Section 2.2. Decontaminant containment preparation and initial system setup occurred during Oct. 19-20. The initial system setup included verifying that all components were onsite, locating a power generator of sufficient capacity, and taking delivery of a temporary office trailer for the process control center.

#### Readiness Demonstration

A readiness demonstration was performed after the aircraft was readied for testing and before processing monitor placement. During Oct. 21-22, the engineering team performed operational testing of the system components, ensuring that the power, air and data distribution systems were functioning properly. An engineering test using only distilled water (no  $\text{H}_2\text{O}_2$  or  $\text{NH}_3$ ) was performed on Oct. 25. A brief, 40 min, VHP test on Oct. 26 verified that a target concentration of 150 ppm could be attained.

### 3.2 Demonstration Testing

The test schedule proceeded as shown in Table 2.

<b>Table 2: C-141 Demonstration Test Schedule</b>					
<b>Date</b>	<b>Decontaminant</b>	<b>Duration (hr)</b>	<b>H<sub>2</sub>O<sub>2</sub> Concentration (ppm)</b>	<b>NH<sub>3</sub> Concentration (ppm)</b>	<b>Agent Surrogates / Simulants</b>
Oct. 27	VHP	1	250	0	BI
Oct. 27	mVHP	1	250	15	BI
Oct. 28	mVHP	1	250	15	BI
Nov. 9-10	VHP	24	250	0	BI, CW and BW
Nov. 12	VHP	5	250	0	BI, CW and BW
Nov. 15	VHP	10.5	250	0	BI, CW and BW
Nov. 16-17	mVHP	24	250	20	BI, CW and BW

#### 3.2.1 Four Step mVHP Process

##### Dehumidification Phase

A stand-alone Munter dehumidifier is normally used to lower interior air humidity to a level < 40%. Dehumidification was not needed because of the low relative humidity (%RH) in Tucson during the tests. Instead, the air handling and exhaust systems blowers were turned on to initiate air circulation. The cargo area vaporizer module pre-heaters were activated to prevent condensation of vapor and the fans were turned on sequentially to increase air circulation.

##### Conditioning Phase

When the internal air was determined to be sufficiently dry, the vaporizers were activated and the fumigant was injected into the aircraft. The addition of a gaseous adjuvant to the vapor phase VHP was achieved through the use of a mass flow controller (Manufacturer: Brooks Instruments, Model No.: MF60S/AC1BB0BA0KA1B1). The concentration of ammonia in the final gaseous mixture (24-hr mVHP run) was approximately 20 ppm. Vapor concentration was brought to the target level of 250 ppm. The dehumidification phase and condition phase for this test program at 250-ppm hydrogen peroxide was approximately 2 hr in duration.

##### Decontamination Phase

The decontamination phase commenced once the vapor concentration target level was achieved. Steady VHP/mVHP concentration was maintained throughout the designated time period of each run. The Ct values for each vaporizer were calculated for each test run.



## Aeration Phase

The aeration phase commenced at the conclusion of the exposure period. At this stage, the vaporizers and heaters were turned off and the blowers left on. The aircraft interior was aerated and the VHP/mVHP fumigant rendered below PEL levels. The hydrogen peroxide was catalytically reduced to oxygen and water. The ammonia, used only during the 24-hr mVHP run, was filtered from the air as it passed through the exhaust system filters. The 5-hr VHP test duration was 10.25 hr. The first three phases were approximately 7 hr in length. The aeration phase was approximately 3.5 hr. Similarly, the 24-hr mVHP test duration was 30 hr. The first three phases were approximately 27 hr in length. The aeration phase was approximately 4 hr. When the interior vapor concentration fell to a safe level, the aircraft doors were opened.

### 3.2.2 Oct. 27-28<sup>th</sup>, VHP and mVHP 1-hr Tests

During the short 1-hr scoping tests, STE personnel placed, recovered, and processed the BIs following fumigation. After the completion of the aeration phase, the indicators were recovered and returned via Federal Express to the STERIS laboratories in Mentor, OH for processing.

### 3.2.3 Nov. 9-10<sup>th</sup>, VHP 24-hr Test

Data retrieval, analysis and inspection of the aircraft identified an aircraft leak that depleted two of the six vaporizers of their hydrogen peroxide supply during the 24-hr VHP run. The hydrogen peroxide container was depleted in Vaporizer 6 at about 18-hr elapsed time and in Vaporizer 5 at about 20-hr elapsed time. The fumigant depletion was attributed to air encroaching from the outside through the leaky aircraft tail, which caused the vaporizers in that area to inject greater amounts of hydrogen peroxide in order to maintain the target concentration in that region. Even after the vaporizers were depleted, however, the concentration in the rear of the aircraft remained above 200 ppm, apparently due to good mixing by the fans.

The BIs and surrogate coupons were processed by ECBC personnel in the mobile microbiological laboratory deployed at the site of the demonstration. The BIs were examined for color change, which indicated successful inactivation. Despite the lower VHP concentration in the rear of the cargo area, the data presented in Section 3 shows that all of the BI's were successfully killed during this test.

The vaporizers were relocated to meet the challenge of the aircraft tail space. Vaporizer modules five and six were relocated closer to the tail of the aircraft to counter aircraft leakage and maintain target fumigant concentration.

Decontamination efficacy is directly related to Ct values, which are derived by multiplying concentration of the fumigant by time of exposure. For this study, the exposure time was achieved by varying time while maintaining near constant



VHP/mVHP fumigant concentration (250 ppm). The Ct values for six vaporizers from this test are presented Table 3.

<b>Table 3: Ct Values for Nov. 9-10th, 24-hour VHP Test</b>		
<b>Vaporizer Number</b>	<b>Internal Monitor A (ppm-hr)</b>	<b>Internal Monitor B (ppm-hr)</b>
1	5767	7528
2	7189	6000
3	7937	5866
4	6165	6990
5	6738	5708
6	5826	5378

#### 3.2.4 Nov. 12<sup>th</sup>, VHP 5-hr Test

This test occurred without difficulty. As discussed in Section 2.2.5, vaporizer module five (Figure 15) was relabeled as unit seven after the 24-hr VHP run. Tables 4 through 6 show the omission of unit five, but present Ct values for unit seven. The Ct values for six vaporizers from this test are presented Table 4.

<b>Table 4: Ct Values for Nov. 12th, 5-hour VHP Test</b>		
<b>Vaporizer Number</b>	<b>Internal Monitor A (ppm-hr)</b>	<b>Internal Monitor B (ppm-hr)</b>
1	1406	1855
2	1823	1473
3	1894	1466
4	1520	1690
6*	1427	1340
7*	1722	1401

#### 3.2.5 Nov. 15<sup>th</sup>, VHP 10.5-hr Test

This test occurred without difficulty. The Ct values for six vaporizers from this test are presented Table 5.

<b>Table 5: Ct Values for Nov. 15th, 10.5-hour VHP Test</b>		
<b>Vaporizer</b>	<b>Internal Monitor A</b>	<b>Internal Monitor B</b>
1	2545	3236
2	3368	2496
3	3205	2528
4	2622	2872
6*	2477	2387
7*	2865	2449

### 3.2.6 Nov. 16-17<sup>th</sup>, mVHP 24-hr Test

This test occurred without difficulty. The Ct values for six vaporizers from this test are presented in Table 6.

<b>Table 6: Ct Values for Nov. 16-17th, 24-hour mVHP Test</b>						
<b>Vaporizer Number</b>	<b>Internal Monitor A (ppm-hr)</b>			<b>Internal Monitor B (ppm-hr)</b>		
	<b>VHP</b>	<b>NH<sub>3</sub></b>	<b>VHP/NH<sub>3</sub></b>	<b>VHP</b>	<b>NH<sub>3</sub></b>	<b>VHP/NH<sub>3</sub></b>
1	5757	124	46.43	7279	175	41.59
2	7360	77	95.58	5724	170	33.61
3	7619	109	69.9	5608	112	50.07
4	5878	143	41.1	6989	155	45.09
6*	5801	106	54.73	5451	146	37.34
7*	6760	93	72.69	5556	123	45.17

## 3.3 Biological Efficacy Testing

### 3.3.1 Coupon Preparation

For biological efficacy testing, the coupons were made from three military-relevant surface materials: glass, bare aluminum, and CARC. The uniformity of the test materials was maintained by obtaining a large quantity of each material. Multiple samples, each measuring 1.3 cm<sup>2</sup>, were cut to order from the same batch of each material by the ECBC Experimental Fabrication shop. The coupons, in sterile glass Petri dishes, were transported from ECBC to the test site in the mobile desert microbiology laboratory. The coupons were removed from the Petri dishes just prior to being inoculated with *G. stearothermophilus* spores.



### 3.3.2 Bacterial Spore Preparation

*G. stearothermophilus* spore stocks were prepared as described by J.L. Dang<sup>5</sup> with some modification. Bacterial spores were harvested from seven to ten day old cultures plated upon Lemko Agar. The spores were washed three times in sterile distilled water (dH<sub>2</sub>O), and collected by centrifugation for 15 min at 1965 rcf x g between washings. The spores were then incubated in 70% (v/v) ethanol for 1 hr; collected by centrifugation; and subsequently incubated in sterile dH<sub>2</sub>O at 73 °C for 1 hr. Spore stocks were titered and stored at 4 °C.

### 3.3.3 Coupon Inoculation

The surface of each coupon was inoculated with  $1 \times 10^7$  bacterial spores in peptone water supplemented with 5% fetal bovine serum as a 10-μL volume. The spore-inoculated coupons were left in a bio safety level two hood until they appeared visibly dry prior to testing.

### 3.3.4 Use of BIs

Commercial and laboratory prepared BIs of *G. stearothermophilus* spores functioned as a confirmatory test for sporicidal effectiveness. The commercial BIs, inoculated to a level of approximately  $10^6$  colony forming units (CFUs), were purchased from two vendors, Apex (ATCC 12980) and STERIS (ATCC 7953). *G. stearothermophilus* was specifically selected for testing since it is a spore forming bacterium that has been identified as the most difficult organism to decontaminate with the VHP technology.

### 3.3.5 Placement of BI Strips and Coupons Prior to Testing

The BIs, sheathed in a Tyvek® pouch, were distributed in replicate throughout the aircraft and then exposed to the VHP antimicrobial. Numerous BIs were suspended from sterile hooks, away from any surfaces that could potentially be contaminated.

### 3.3.6 Coupon and BI Analysis

Following exposure to the VHP antimicrobial, the BIs and one of each type of inoculated coupon from each location were placed in a 5-mL volume of sterile growth medium for viability testing. The cultures were incubated at 55 °C for a period of seven days for BIs and 24 hr for coupons. Lack of turbidity following incubation was considered a non-viable sample. If turbidity was detected, coupons were processed for enumeration. Coupons were placed in 5 ml recovery media and sonicated. After sonication, 10 μL of 1% Antifoam 289 (Sigma Aldrich Chemical Co.) was added, and then the suspension was vortexed. Samples were then serially diluted and pour plated (1 mL/plate) in triplicate with appropriate growth medium, allowed to solidify, and incubated at 55°C overnight. Resultant colonies were enumerated using a Q-Count Colony Counter (Spiral Biotech). Experimental manipulations such as BI and coupon assays and enumeration were conducted in the mobile desert microbiology laboratory.



<b>Table 7: Biological Indicator Locations*</b>			
<b>BI</b>	<b>Y* (Inches)</b>	<b>X** (Inches)</b>	<b>Notes</b>
1, 2	72	300	
3, 4	-12	550	Inside open box on wall
5, 6	72	670	On sensor
7, 8	48	900	On ceiling
9, 10	72	900	On sensor
11, 12	144	900	Near exhaust
13, 14	72	1150	Near inlet on vaporizer
15, 16	72	1530	On rear pressure door
17, 18	144	1320	On upper bar of door
19, 20	72	1240	On sensor

\*For Engineering Runs, October 27-28

\*\*Per plane markings

### 3.3.7 Biological Efficacy

The BI results are tabulated in Table 8. Coupon challenge results are presented in bar graph format in Figure 18. Six-hundred *G. stearothermophilus* spore laden BI strips were used in the demonstration testing. Out of 600, 597 BI strips were rendered completely non-viable. The three viable BIs are associated with the 5-hr VHP treatment. Laboratory prepared spore laden coupons in a 5% serum solution comprised glass, aluminum, and CARC. These were deployed at the same six locations within the aircraft for each of the four VHP/mVHP runs.

Table 8: Biological Indicator Results									
BI	Location	Nov. 9-10 24-hr VHP		Nov. 12 5-hr VHP		Nov. 15 10.5-hr VHP		Nov. 16-17 24-hr mVHP	
		STERIS	Apex	STERIS	Apex	STERIS	Apex	STERIS	Apex
1	Galley, port alcove	-	-	-	-	-	-	-	-
2	Port - 370, mid-height			-	-	-	-		
3	Port - 430, mid-height	-	-	-	-	-	-	-	-
4	Port - 530, box behind sensor 2A			-	-	-	-		
5	Port - 650, emergency exit port hole	-	-	-	-	-	-	-	-
6	Port - 740, aft on rib # 23			-	-	-	-		
7	Port - rib, 830	-	-	-	-	-	-	-	-
8	Port - 850, indentation			-	-	-	-		
9	Port - 950, at horn	-	-	-	-	-	-	-	-
10	Port - 1050 - mid height			-	-	-	-		
11	Port - 1050, aft side of rib	-	-	-	-	-	-	-	-
12	Port - 1080, indent			-	-	-	-		
13	Port - 1150, mid-height	-	-	-	-	-	-	-	-
14	Port - 1200, emergency exit #46			-	-	-	-		
15	Port - 1310, mid-height	-	-	-	-	-	-	-	-
16	Port - 1370, mid-height			-	-	-	-		

**Table 8: Biological Indicator Results (continued)**

BI	Location	Nov. 9-10 24-hr VHP		Nov. 12 5-hr VHP		Nov. 15 10.5-hr VHP		Nov. 16-17 24-hr mVHP	
		STERIS	Apex	STERIS	Apex	STERIS	Apex	STERIS	Apex
17	Port - 1410, control panel	-	-	-	-	-	-	-	-
18	Port - aft rib			-	-	-	-		
19	Port - base of sensor 6B	-	-	-	-	-	-	-	-
20	Port - aft locking mechanism			-	-	-	-		
21	Port - aft bulkhead	-	-	-	-	-	-	-	-
22	Center of ramp			-	-	-	-		
23	Starbord - behind sensor, rear	-	-	-	-	-	-	-	-
24	Starbord - bulkhead, rear			-	-	-	-		
25	Starbord - 4th rib, deployment area, aft surface	Not Recovered		-	-	-	-	-	-
26	Starbord - 2nd rib, deployment area			+	-	-	-		
27	Starbord - steel mechanism	-	-	-	-	-	-	-	-
28	Starbord-1410, mid-height, fire suppression equip. alcove			-	-	-	-		
29	Starbord - 140, mid-height	-	-	-	-	-	-	-	-
30	Starbord - 1360, mid-height			-	-	-	-		
31	Starbord - 1310, mid-height	-	-	-	-	-	-	-	-
32	Starbord - 1220, mid-height			-	-	-	-		
33	Starbord-1160, emer. exit window in door leaning against interior bulkhead	-	-	-	-	-	-	-	-
34	Starbord - 1100, fore surface of pipe			-	-	-	-		
35	Starbord, 1050 mid height	-	-	-	-	-	-	-	-
36	Starbord - 950 mid-height			-	-	-	-		
37	Starbord - 870, mid-height	-	-	-	-	-	-	-	-
38	Starbord - 830, in alcove			-	-	-	-		
39	Starbord - 750, on oxygen box	-	-	-	-	-	-	-	-
40	Starbord - on oxygen sensor unit			-	-	-	-		
41	Starbord - 650, porthole	-	-	-	-	-	-	-	-



**Table 8: Biological Indicator Results (continued)**

BI	Location	Nov. 9-10 24-hr VHP		Nov. 12 5-hr VHP		Nov. 15 10.5-hr VHP		Nov. 16-17 24-hr mVHP	
		STERIS	Apex	STERIS	Apex	STERIS	Apex	STERIS	Apex
42	Starbord - 530, mid-height			-	-	-	-		
43	Starbord - 460, aft interior panel	-	-	-	-	-	-	-	-
44	Starbord - 450, inside box			-	-	-	-		
45	Starbord - 390, on shelf behind sensor	-	-	-	-	-	-	-	-
46	Starbord - 370, alcove			-	-	-	-		
47	Starbord - 350, under shelf	-	-	-	-	-	-	-	-
48	Forward bulkhead, starbord side			-	-	-	-		
49	Starbord - 320, under MGD kit	-	-	-	-	-	-	-	-
50	Forward bulkhead, under loadmaster log			-	-	-	-		
51	Forward, under steps to flight deck	-	-	-	-	-	-	-	-
52	Port, sensor			-	-	-	-		
53	Starbord, back of sensor	-	-	-	-	-	-	-	-
54	Sensor 2A			-	-	-	-		
55	Sensor 2B	-	-	-	-	-	-	-	-
56	Sensor 3A			-	-	-	-		
57	Sensor 3B	-	-	-	-	-	-	-	-
58	Sensor 4A			-	-	-	-		
59	Sensor 4B	-	-	-	-	-	-	-	-
60	Sensor 5A			-	-	-	-		
61	Sensor 5B	-	-	-	-	-	-	-	-
62	Sensor 6B, starbord			-	-	-	-		
63	Sensor 6B, port	-	-	-	-	-	-	-	-
64	Ceiling- center, near ramp			-	-	-	-		
65	Aft side of gear pin box, forward section	-	-	-	-	-	-	-	-
66	Starbord - 1220, under step			-	-	-	-		

**Table 8: Biological Indicator Results (continued)**

BI	Location	Nov. 9-10 24-hr VHP		Nov. 12 5-hr VHP		Nov. 15 10.5-hr VHP		Nov. 16-17 24-hr mVHP	
		STERIS	Apex	STERIS	Apex	STERIS	Apex	STERIS	Apex
67	1390 ceiling	-	-	-	-	-	-	-	-
68	Under Vaporizer 6			-	-	-	-		
69	Floor, Vaporizer 7	-	-	-	-	-	-	-	-
70	Under Vaporizer 5			-	-	-	-		
71	Under Vaporizer 4	-	-	+	-	-	-	-	-
72	Vaporizer #2, top			-	-	-	-		
73	Ceiling, center, 510	-	-	-	-	-	-	-	-
74	Ceiling, center, 650			-	-	-	-		
75	Hung above platform, port side	-	-	-	-	-	-	-	-
76	Ceiling, port, 860			-	+	-	-		
77	Ceiling, center, 1040	-	-	-	-	-	-	-	-
78	Ceiling, center, 1260			-	-	-	-		
79	On platform, overhead, forward section	-	-	-	-	-	-	-	-
80	On platform, overhead, aft section			-	-	-	-		
81	On floor forward side of Generator 1	-	-	-	-	-	-	-	-
82	Port, 490, bottom of step along wall			-	-	-	-		
83	Starbord, bottom edge of wall under open flap of step	-	-	-	-	-	-	-	-
84	Port, base of second fan			-	-	-	-		
85	Behind lowest step of ladder on forward bulkhead	-	-	-	-	-	-	-	-
86	Bottom, aft frame of Generator 3			-	-	-	-		
87	Port, 750, on wall above step	-	-	-	-	-	-	-	-
88	Aft port deployed, area over bulkhead			-	-	-	-		
89	900, aft side of hand pump box	-	-	-	-	-	-	-	-
90	Port, 800, lower edge of step			-	-	-	-		
91	Port, 990, on cap embedded into wall near 4th fan	-	-	-	-	-	-	-	-

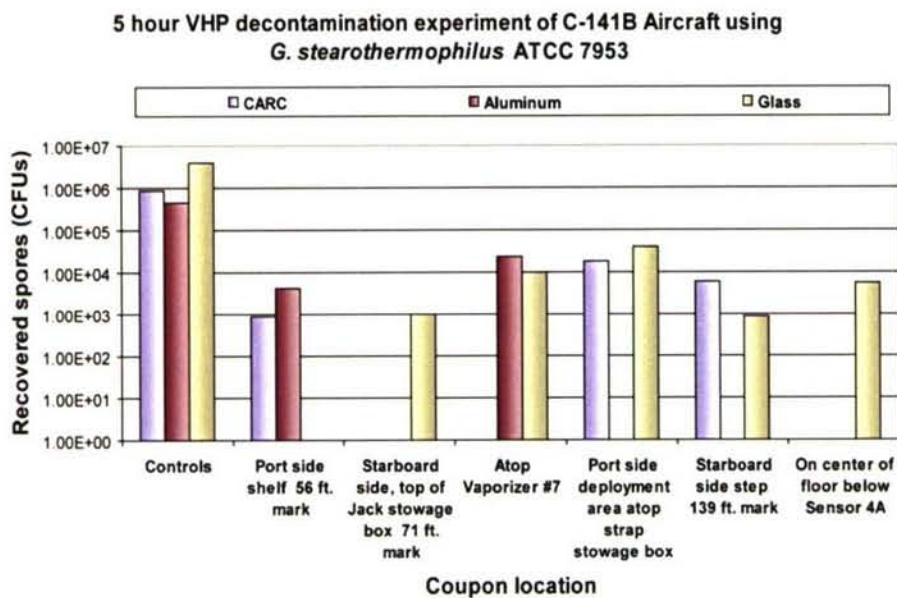
**Table 8: Biological Indicator Results (continued)**

BI	Location	Nov. 9-10 24-hr VHP		Nov. 12 5-hr VHP		Nov. 15 10.5-hr VHP		Nov. 16-17 24-hr mVHP	
		STERIS	Apex	STERIS	Apex	STERIS	Apex	STERIS	Apex
92	Starbord 1040, on step			-	-	-	-		
93	Starbord, 1110, on pipe	-	-	-	-	-	-	-	-
94	Starbord deployment area, alcove, forward			-	-	-	-		
95	Ramp, lower edge, starbord	-	-	-	-	-	-	-	-
96	Front control cabinet deployment area			-	-	-	-		
97	Aft overhead in circle, left side	-	-	-	-	-	-	-	-
98	Port deployment area aft inside box			-	-	-	-		
99	Aft overhead in circle, right side	-	-	-	-	-	-	-	-
100	Starbord engine oil box deployment area			-	-	-	-		



**Figure 18: *G. stearo*thermophilus ATCC 7953VHP Exposure Test Results**

**a) 5-Hour VHP Decontamination Experiment**



**b) 10.5-Hour VHP Decontamination Experiment**

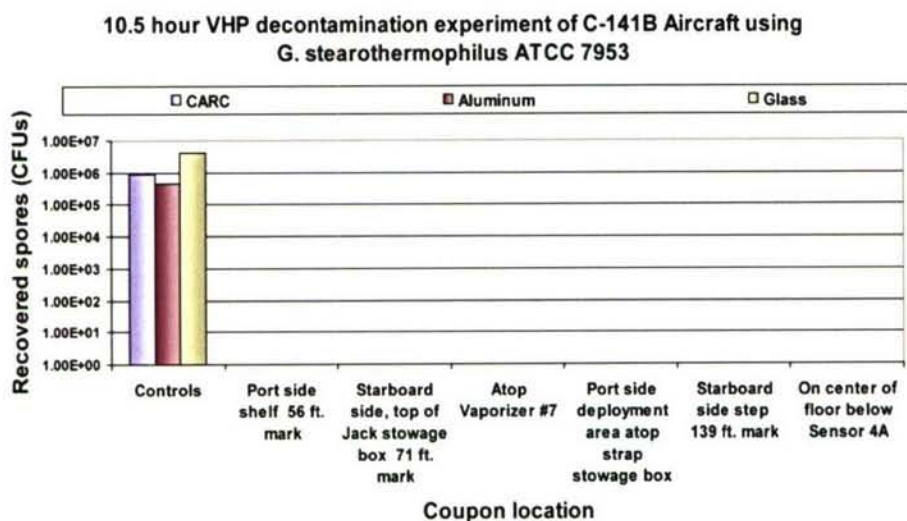
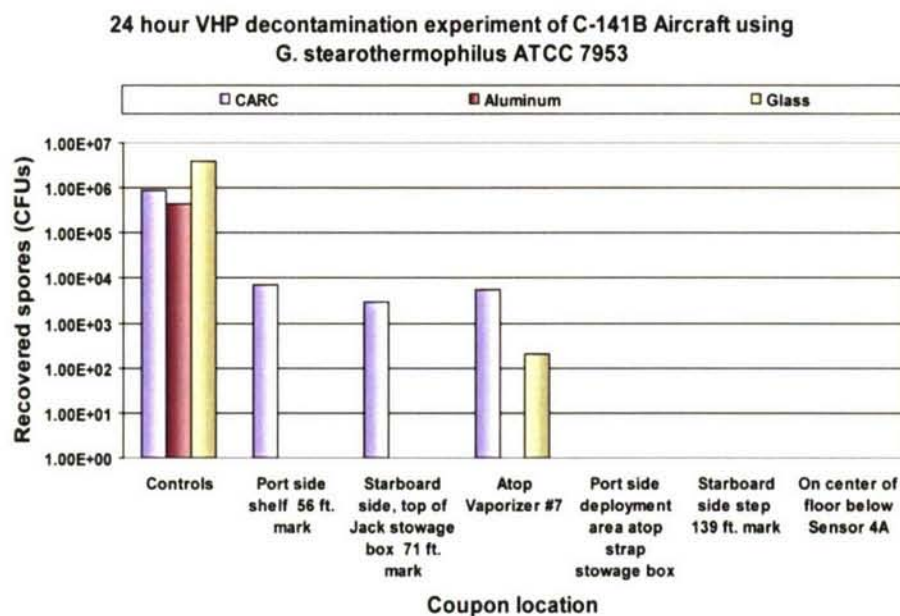
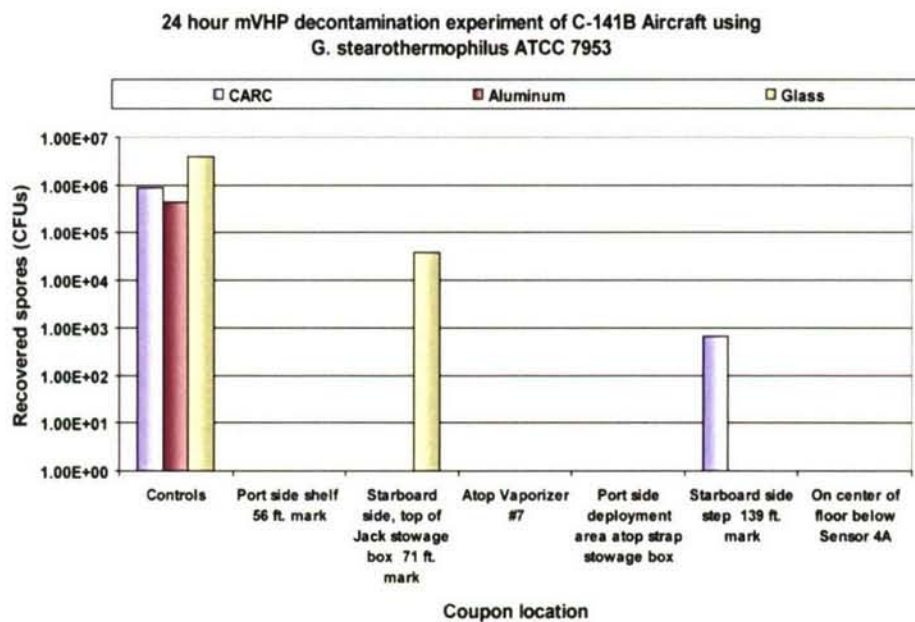


Figure 18: *G. stearotherophilus* ATCC 7953VHP Exposure Test Results (continued)

c) 24-Hour VHP Decontamination Experiment



d) 24-Hour mVHP Decontamination Experiment



### 3.4 Chemical Efficacy Testing

#### 3.4.1 Sample Preparation

VHP/mVHP technology evaluation of chemical agent simulant decontamination efficacy was conducted on bare and CARC-painted aluminum coupons. Two-inch diameter circles were punch-cut from Al 2024 aluminum stock to make the coupons. The coupons were polished to remove burrs and rough edges. They were then separated into two groups. The aluminum surface of the first group was not altered in any way. The second group of coupons was painted with a military grade of polyurethane paint, CARC, and finished in accordance with (IAW) 4.9 MIL-STD-171, per MIL-C-53039A, #383 green. Prior to use, all coupons were inspected for irregularities, cleaned with 2-propanol, dried at 40 °C in an oven and stored in a clean environment.

Chloroethyl phenyl sulfide (CEPS) mimics the oxidative conversion to the sulfoxide product of HD.<sup>6</sup> CEPS was selected as the chemical simulant for the C-141B test. The CEPS was purchased from Lancaster (lot # R 20/21/22/36/38) and used in the liquid form it was received. HD was used in several correlation studies with the CEPS. The HD was chemical agent standard analytical reference material (CASARM) and was supplied by the ECBC Chemical Agent Transfer Facility.

The test coupons were contaminated to a density of 0.82 g/m<sup>2</sup>. The CEPS was applied by pipetting 0.5 µL drops in a uniform repetitive distribution pattern onto the coupon surfaces. The coupons were then placed in a plastic storage container and covered to prevent the evaporation of the simulant. After a 1-hr dwell period at ambient temperature, the coupons were removed from the storage container and placed in the aircraft at one of three predetermined locations within the fuselage. The fuselage location station, as marked on the bulkhead of the fuselage and the height from the fuselage floor are indicated in Table 9.

**Table 9: Fuselage Settings**

<b>Sample Position</b>	<b>Location (Station)</b>	<b>Height (Inches)</b>
1	670	8
2	890	48
3	1230	72

#### 3.4.2 Chemical Warfare Agent Simulant Vapor Analysis

Vapor cups were prepared for residual chemical agent surrogate analysis of the decontaminated coupons. The cups, fashioned from seamless tin cans with lids (3-in. diameter x 1-in. height), were purchased from McMaster-Carr. Two 7/16-in. holes were punched in each lid to accept two stainless steel, ¼-inch fitting, male bulkhead Swagelok® connectors obtained from the Baltimore Valve Company. A ¼-in. Teflon® furrell was inserted into each fitting to accept a depot area air monitoring system (DAAMS) tube at one end of the cup and a charcoal filter at the other (Figure 19).



The coupons were removed from the aircraft following the indicated decontamination period and immediately placed in the vapor cups for analysis. Vacuum lines were instantly connected to the exit ports and the timed cup evacuation was started. Ambient air, conditioned through a charcoal (BPL 30 to 40 mesh) trap, was forced into the cups and made to flow at a pre-set rate of 80 mL/min. The air stream leaving the cup exited through a DAAMS tube to absorb any CEPS that off-gassed (volatilized) from the test coupon. Each sample period lasted 60 min to yield a total volume of air at 4,800 cm<sup>3</sup>. The DAAMS tubes were then removed and stored in capped glass containers until analyzed.

The CEPS concentration was determined by a gas chromatograph (GC) equipped with a flame photometric detector in the sulfur mode. Each DAAMS tube was inserted into Dynatherm, which is designed to thermally desorb the analyte from the DAAMS Tenax solid sorbent and transfer the vapor into the coupled Agilent 6852 GC inlet. The column was a 0.25 mm x 15 m DB-210 with N<sub>2</sub> carrier at 10 psi. The initial column temperature of 60 °C was held for one minute and then ramped to 200 °C at 45 °C/min. The injector and detector temperatures were 250 °C and 300 °C, respectively.

A quality control process was employed throughout the analysis of the test samples. Quality Process and Quality Lab samples were taken IAW the US Army Technical Escort Unit's Quality Assurance Plan (US Army TEU Aberdeen Proving Ground, Jan 2004).



### 3.4.3 Correlation Studies (CEPS and HD)

The use of CEPS as suitable HD simulant was validated in a side-by side study. In a previous study, CEPS and HD spiked coupons were compared side-by-side within an environment of VHP. In the present study, two correlations were run.

The first correlation study was conducted to simply determine the recovery of CEPS compared to that of HD from an aluminum surface. The bottom of the inside of a vapor cup was measured into two equal sections and a line was drawn with a grease pencil down the middle. Both the agent simulant and agent were concentrated at 7.2 ng (4  $\mu$ L of a 1.8 ng/ $\mu$ L solution of analyte in hexane) and each was applied to one section of the bottom of the cup. The lid to the cup was tightly fastened and a vacuum flow of 400 mL/min of ambient air was introduced into the cup for 6 min, which yielded the same concentration for a one Time Weighted Average (TWA) of HD (400 mL/min x 6 min x 0.003 mg/m<sup>3</sup>). Compared to a three-point calibration curve, the recovery for CEPS and HD was 85% and 95%, respectively.

The second correlation study attempted to measure the decay of both CEPS and HD on aluminum and CARC painted surfaces. This study concluded that CEPS is more resistant to the VHP/mVHP fumigant than HD.

### 3.4.4 Results of the C-141B Test Coupons

The post-decontamination CEPS vapor hazard was measured for each coupon. The results are compared against the one TWA vapor hazard value for HD. The one TWA HD vapor hazard value is 0.003 mg/m<sup>3</sup>. The results from the 5-hr VHP test, Table 10 and Figure 23, illustrate that CEPS was not detected on any of the surfaces except for one CARC replicate located at location three. The CEPS results are below the corresponding one TWA for HD.



**Table 10: Chemical Agent Simulant GC Results, 5-hr VHP Test Nov. 12**

<b>Substrate</b>	<b>Sample Location</b>	<b>VHP Exposure Time, hours</b>	<b>Mass Found (ng)</b>	<b>CEPS Concentration mg/m3</b>	<b>Sample ID</b>
Aluminum	1	5	0.00	0.00000	111504-1410
	1	5	0.00	0.00000	111504-1435
	1	5	0.00	0.00000	111504-1445
	1	5	0.00	0.00000	111504-1500
	2	5	0.00	0.00000	111504-1320
	2	5	0.00	0.00000	111504-1330
	2	5	0.00	0.00000	111504-1345
	2	5	0.00	0.00000	111504-1400
	3	5	0.00	0.00000	111504-1230
	3	5	0.00	0.00000	111504-1245
	3	5	0.00	0.00000	111504-1310
CARC	1	5	0.00	0.00000	111504-1510
	1	5	0.00	0.00000	111504-1525
	1	5	0.00	0.00000	111504-1535
	1	5	0.00	0.00000	111504-1550
	2	5	0.00	0.00000	111504-1600
	2	5	0.00	0.00000	111504-1615
	2	5	0.00	0.00000	111504-1645
	2	5	0.00	0.00000	111504-1650
	3	5	2.82	0.00059	111504-1715
	3	5	0.00	0.00000	111504-1730
	3	5	0.00	0.00000	111504-1705



The results from the 10.5-hr VHP test, Table 11 and Figure 20, illustrate that a low CEPS concentration was detected on both the bare aluminum and CARC painted coupons for this run. Except for three CARC coupons, the CEPS concentration is below the corresponding one TWA for HD.

<b>Table 11: Chemical Agent Simulant GC Results, 10-hr VHP Test Nov. 15th</b>					
<b>Substrate</b>	<b>Sample Location</b>	<b>VHP Exposure Time, hours</b>	<b>Mass Found (ng)</b>	<b>CEPS Concentration mg/m3</b>	<b>Sample ID</b>
Aluminum	1	10	5.30	0.00110	111604-2359
	1	10	7.68	0.00160	111604-2408
	1	10	10.11	0.00211	111604-2418
	1	10	9.69	0.00202	111604-2427
	2	10	12.18	0.00254	111604-2319
	2	10	11.24	0.00234	111604-2329
	2	10	9.56	0.00199	111604-2340
	2	10	7.26	0.00151	111604-2349
	3	10	9.72	0.00203	111604-2258
	3	10	9.19	0.00191	111604-2309
CARC	1	10	8.05	0.00168	111604-2014
	1	10	8.22	0.00171	111604-2030
	1	10	7.54	0.00157	111604-2043
	2	10	6.24	0.00130	111604-2104
	2	10	10.99	0.00229	111604-2115
	2	10	27.49	0.00573	111604-2125
	3	10	29.05	0.00605	111604-2152
	3	10	16.98	0.00354	111604-2203
	3	10	8.62	0.00180	111604-2214

The results from the 24-hr VHP tests, Tables 12 and 13, illustrate that a low CEPS concentration was detected on both the bare aluminum and CARC painted coupons for this run. Except for the CARC coupons from position three, the CEPS concentration is below the corresponding one TWA for HD.

<b>Table 12: Chemical Agent Simulant GC Results, 24-hr VHP Test, Nov. 9-10</b>					
<b>Substrate</b>	<b>Sample Location</b>	<b>VHP Exposure Time, hours</b>	<b>Mass Found (ng)</b>	<b>CEPS Concentration mg/m3</b>	<b>Sample ID</b>
Aluminum	1	24	0.00	0.00000	111404-1000
	1	24	1.80	0.00038	111404-0930
	1	24	0.00	0.00000	111404-0900
	1	24	1.90	0.00040	111404-0845
	2	24	0.00	0.00000	111404-1050
	2	24	0.00	0.00000	111404-1030
	2	24	0.00	0.00000	111404-1230-F
	2	24	0.00	0.00000	111404-1245
	3	24	1.84	0.00038	111404-1300
	3	24	1.99	0.00041	111404-1310
	3	24	6.19	0.00129	111404-1315
CARC	1	24	1.78	0.00037	111404-0945
	1	24	0.00	0.00000	111404-0915
	1	24	0.00	0.00000	111404-1100
	1	24	2.10	0.00044	111404-0832
	2	24	1.76	0.00037	111404-1035-F
	2	24	1.96	0.00041	111404-1117-F
	2	24	8.00	0.00167	111404-1130
	2	24	2.03	0.00042	111404-1140
	3	24	0.00	0.00000	111404-1155
	3	24	0.00	0.00000	111404-1205
	3	24	2.00	0.00042	111404-1220

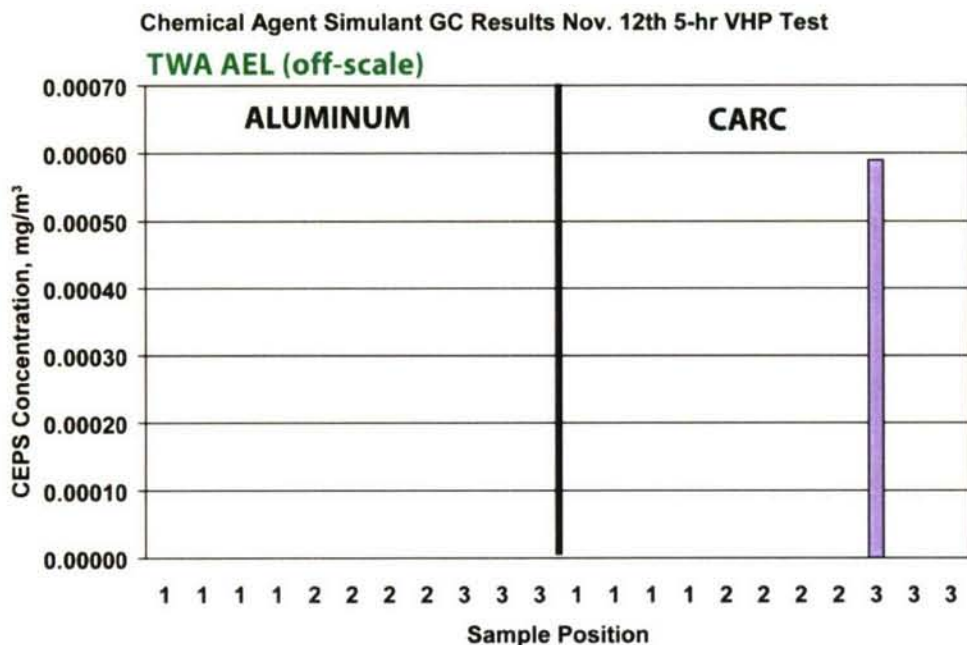
**Table 13: Chemical Agent Simulant GC Results, 24-hr mVHP Test, Nov. 16-17**

<b>Substrate</b>	<b>Sample Location</b>	<b>VHP Exposure Time, hours</b>	<b>Mass Found (ng)</b>	<b>CEPS Concentration mg/m3</b>	<b>Sample ID</b>
Aluminum	1	24	19.26	0.00800	111704-2100
	1	24	2.21	0.00090	111704-2134
	1	24	11.15	0.00460	111704-1752
	1	24	43.74	0.01820	111704-1803
	2	24	6.80	0.00280	111704-2110
	2	24	13.59	0.00570	111704-2112
	2	24	26.11	0.01090	111704-2040
	2	24	20.03	0.00830	111704-2029
	3	24	30.15	0.01260	111704-2144
	3	24	31.50	0.01310	111704-2203
	3	24	22.95	0.00960	111704-1852
CARC	1	24	28.63	0.01190	111704-2111
	1	24	18.99	0.00790	111704-1920
	1	24	16.89	0.00700	111704-1934
	1	24	65.33	0.02720	111704-2040
	2	24	32.41	0.01350	111704-1805
	2	24	19.65	0.00820	111704-1843
	2	24	45.26	0.01890	111704-1900
	2	24	17.18	0.00720	111704-1909
	3	24	6.56	0.00270	111704-2145
	3	24	15.63	0.00650	111704-1804
	3	24	15.75	0.00660	111704-1831

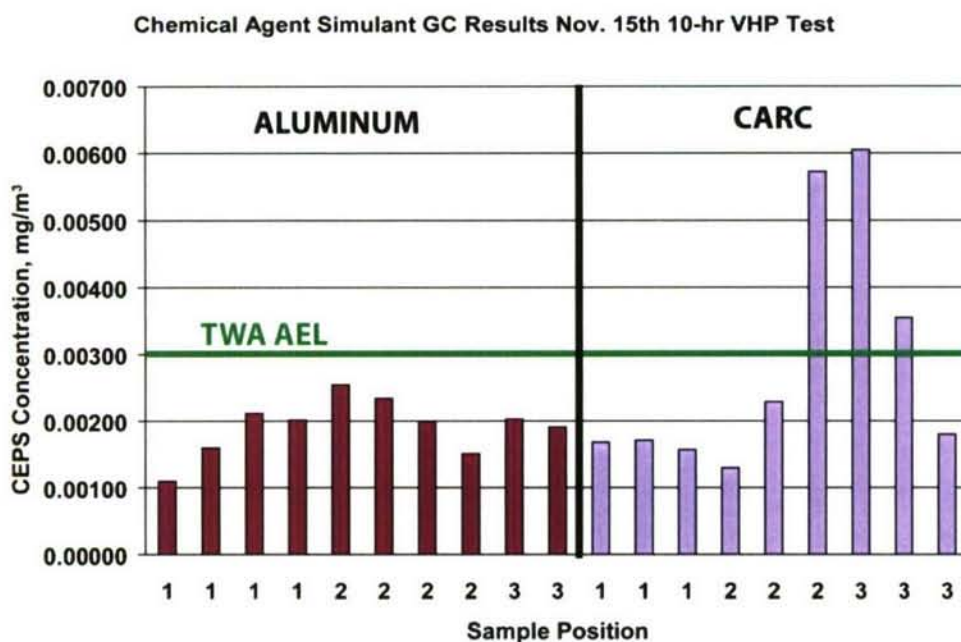


**Figure 20: Chemical Agent Simulant GC Test Results**

a) 5-Hour VHP Decontamination Experiment



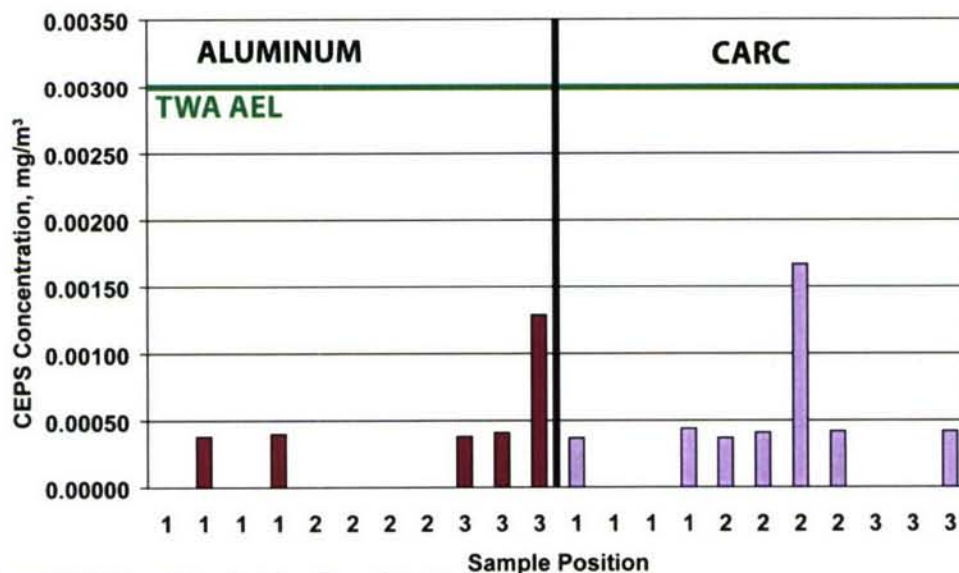
b) 10.5-Hour VHP Decontamination Experiment



**Figure 20: Chemical Agent Simulant GC Test Results (Continued)**

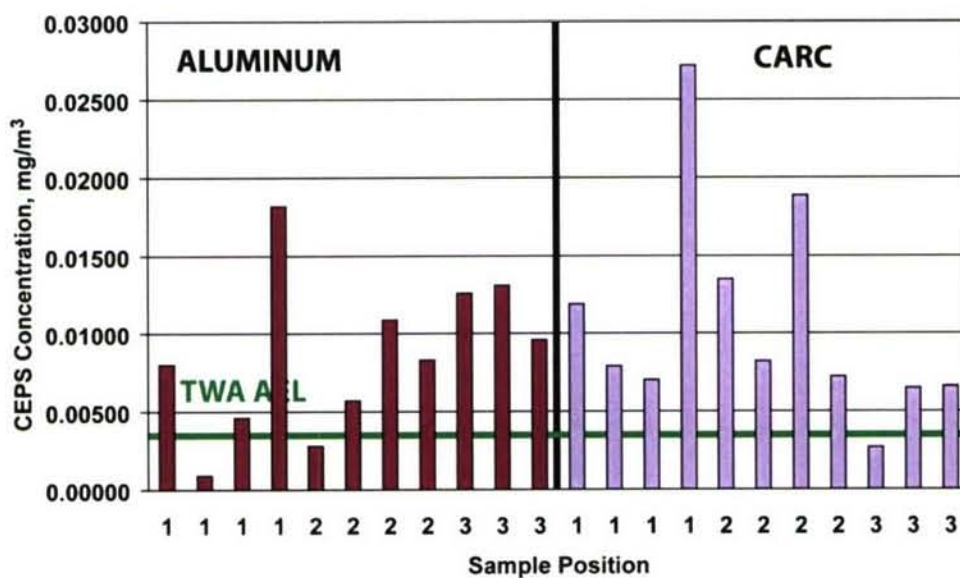
c) 24-Hour VHP Decontamination Experiment

Chemical Agent Simulant GC Results Nov. 9-10th 24-hr VHP Test



d) 24-Hour mVHP Decontamination Experiment

Chemical Agent Simulant GC Results Nov. 16-17th 24-hr mVHP Test



The masses and concentrations of CEPS detected in the 10.5-hr (Table 8) and second 24-hr (Table 9) runs are inconsistent in the 5-hr (Table 7) with the first 24-hr VHP run (Table 6). The results of both the first 24-hr test and the 5-hr test indicated that the concentrations of CEPS were below the detectable concentration limit on all substrates and sample locations. This discrepancy can be explained from the preparation of the vapor cup set-up. The cans used to fashion the vapor cups and Swagelok® connectors employed during the first two tests were never used prior to the test. During the 10.5-hr and second 24-hr test, the connectors were re-used. An attempt was made to clean each of the connectors in the field using acetone and then baking them in the GC oven over night at 120<sup>0</sup>C. Unfortunately, the issue with cross-contamination was not discovered until well into the analysis of the second 24-hr test, due to a lag time required to analyze all of the DAAMS tubes.

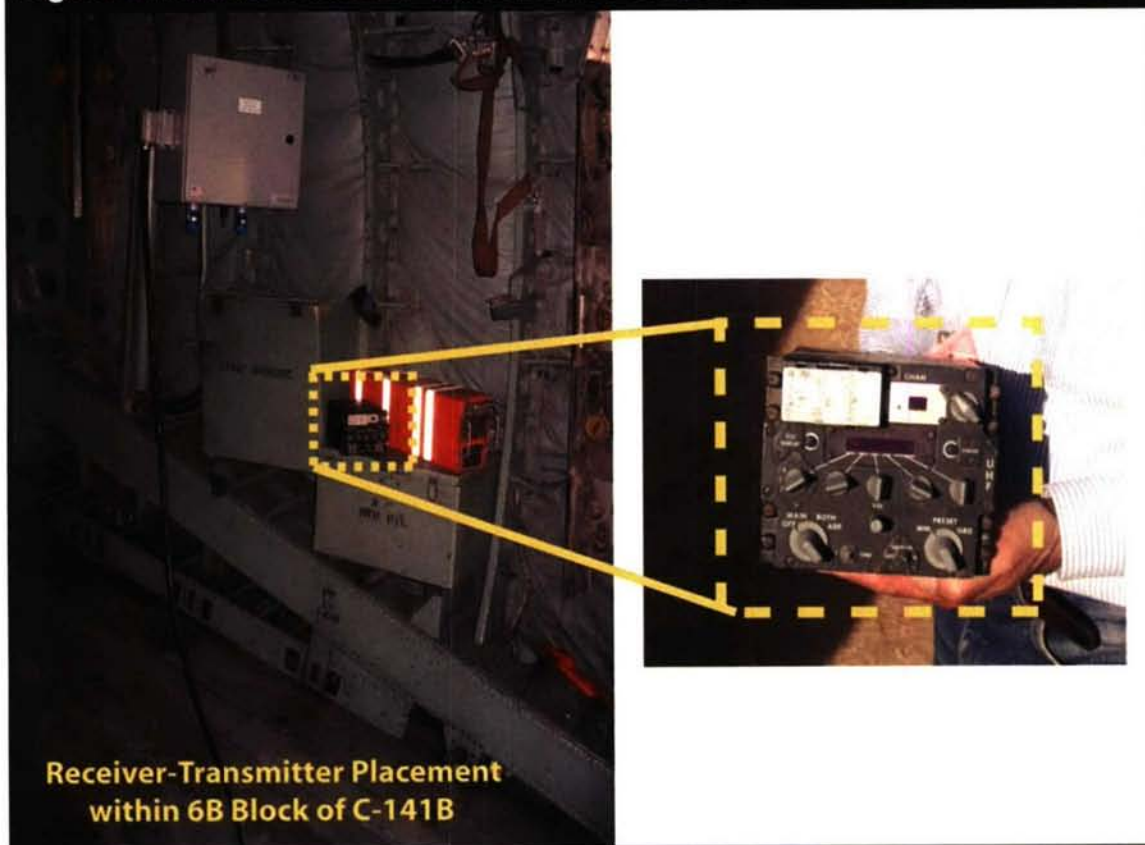
Once this issue was discovered a blank was run to determine if in fact cross-contamination could occur with re-using the connectors. Three connectors were selected at random and then cleaned using a procedure similar to that was for cleaning and re-using connectors during the test. The connectors were washing 2X in acetone and dried in the GC over overnight at 120 °C. Attached to a new vapor can and 1-hr air sample was pulled through the DAAMS tube and then analyzed. Small amounts of CEPS were detected.

### 3.5 Materials and Sensitive Equipment Compatibility

A new PC desktop computer and a Web Cam were exposed to mVHP for 34 hr to test sensitive equipment compatibility to mVHP. The computer and Web Cam were powered and running during testing. A new digital camera received 10 hr of exposure. A radio receiver-transmitter was placed in the tail section of the aircraft near Internal Monitor 6B during the 5-, 10.5-, and 24-hr runs (Figure 21). The transmitter was fully operational during the test runs. Preliminary sensitive equipment fumigant compatibility results demonstrated that no decontamination process adverse effects from the VHP/mVHP exposure were experienced. In addition, there was no loss of preexisting electronic data and each test article functioned before and after fumigant exposure.



**Figure 21: Radio Receiver-Transmitter Placement**



Furthermore, the modular mVHP units have been subjected to more than 200 cumulative hours of fumigant exposure during the building and aircraft demonstrations. Each unit contains an electronic control module and consists of many components representing a variety of materials. No degradation of performance has been observed in any of the components.

### 3.6 Power Consumption

Power consumption of two decontamination runs, the 5-hr VHP exposure and the 24-hr mVHP exposure, was tracked using the Power Monitor model PM820 and the SMS System Manager Software, version 3.3.2.2. Both the system and the software package are manufactured by SquareD.

#### 3.6.1 Weather Conditions

As recorded by the National Weather Service, the weather conditions in Tucson during the time of the two runs are presented in Table 14.

<b>Table 14: Weather Conditions during Decontamination Demonstration Runs</b>			
<b>Decon Test</b>	<b>5-hour VHP</b>	<b>24-hour mVHP</b>	
<b>Date</b>	<b>Nov. 12, 2004</b>	<b>Nov. 16, 2004</b>	<b>Nov. 17, 2004</b>
Temperature, °F			
Maximum	66 at 12:37 pm	68 at 4:07 pm	69 at 4:01 pm
Minimum	46 at 11:51 pm	46 at 7:26 pm	44 at 7:22 pm
Average	56	57	57
Relative Humidity, %			
High	80	86	83
Lowest	31	37	38
Average	56	62	61
Average Wind Speed, MPH	7.3	5.7	6.0

#### 3.6.2 Total Power Consumption

The total power used in each of the two runs is presented in Table 15. In addition to the designated decontamination period for each run, 5-6 hr were needed for the dehumidification, conditioning, and aeration phases. Therefore, the total duration run includes the total time taken to complete all four phases of the process.

<b>Table 15: Total Power Usage for Two Decontamination Demos</b>		
<b>Date</b>	<b>Nov. 12, 2004</b>	<b>Nov. 16-17, 2004</b>
<b>Decon Phase</b>	<b>5-hour VHP</b>	<b>24-hour mVHP</b>
Total Duration, All Phases	10.25 hr	30 hr
Real Power Used, kWH	456	1948
Total Power Used, kWH	473	1982



The 24-hr mVHP run showed approximately four times the power consumption of the 5-hr run. This was probably due to the predominance of the heaters in power consumption. About 6 hr of the Nov. 12 run required heaters, compared to the 25 hr required by the Nov. 16-17 run. The power consumption during the aeration phase of both runs was of comparable length as only the blowers were activated then. Another factor in the 24-hr mVHP run was the low nighttime temperature, which required more heat input to maintain the necessary conditions to prevent vapor condensation. To increase heat generation, the heater of the desiccant dryer in the dehumidification system was activated between midnight and 8 AM. The heater required an additional 50 kW of power (Figure 24a, blue trace). The temperature inside the aircraft during the run was calculated as the average of the 12 sensors distributed throughout the interior (Figure 24a, yellow trace, with standard deviation indicated). The high at approximately 4 PM on Nov. 16 matches the external temperature high as recorded by the National Weather Service, and to a correspondingly lower power requirement from the system heaters. Once the pre-heaters reached maximum capacity, power consumption remained steady at just under 60 kW. However, since the temperature continued to fall during the night, the supplemental desiccant heater was in constant use.

### 3.6.3 Power Consumption of System Components

Power consumption of individual system components was determined during the dehumidification and conditioning phases as the components were activated sequentially. The results for the 24-hr run conditioning phase are provided in Table 16.

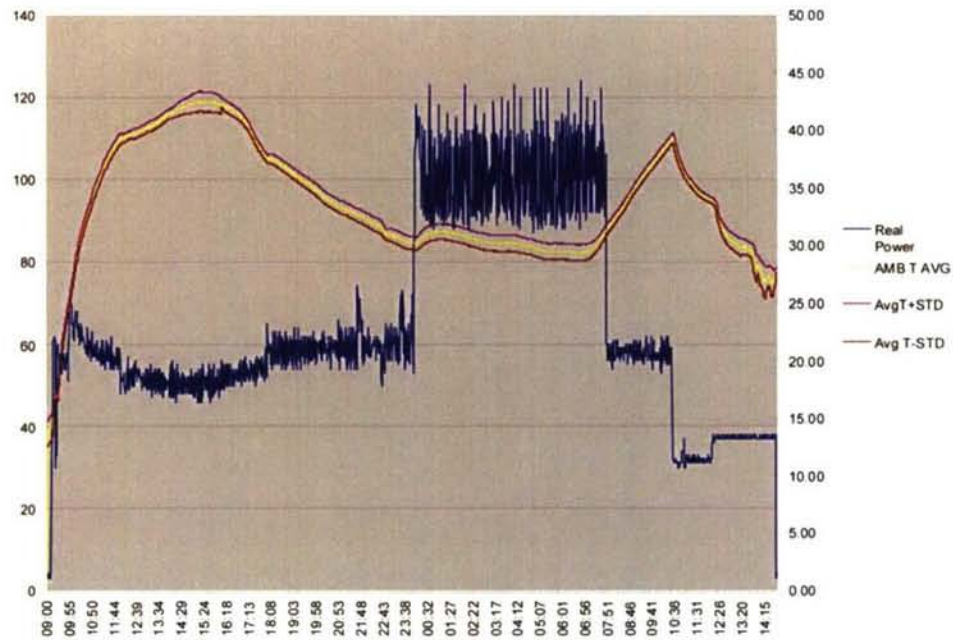
<b>Table 16: Approximate Power Consumption by Components</b>	
<b>System Component</b>	<b>kW</b>
Blowers	33 kW
Pre-heaters	2.3 kW each
Dehumidifier	40 kW

In Figure 22b, real power is indicated by the heavy red line. On Nov. 16, from 9:10 to 9:13 AM, the Air Handling and Exhaust systems were activated and brought to full load, drawing approximately 33 kW. At 9:15 AM, the 12 pre-heaters, two per vaporizer module, were turned on, bringing the power usage to 61 kW. The pre-heaters were temporarily shut down at 9:20 AM, at which point, power again fell to 33 kW, representing the Air Handling and Exhaust system blowers. The vaporizers were activated at approximately 9:50 AM, and after initial fluctuations, power consumption remained between 50 and 60 kW throughout the daytime portion of the decontamination exercise. Later fluctuations represent pre-heater cycles. The maximum power consumption of approximately 100 kW (with spikes to 123 kW) experienced by the system occurred between midnight and 8:00 AM of Nov. 17 while the additional heater in the dehumidification system was operating.

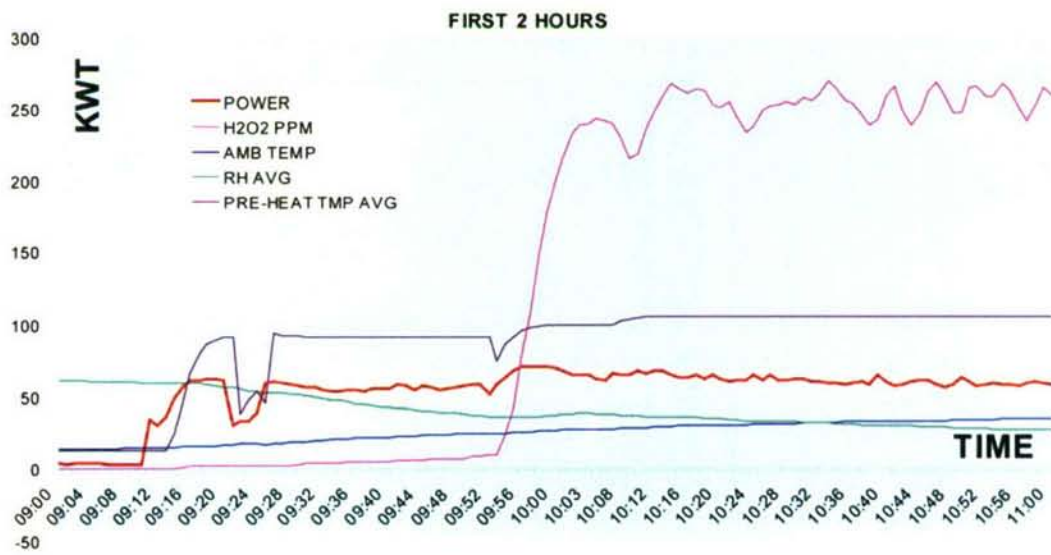


Figure 22: Power Consumption

a) 24-Hour VHP Decontamination Experiment Temperature and Power Consumption



b) Initial Conditioning Phase of 24-Hour Run



### 3.7 Hydrogen Peroxide Consumption

The hydrogen peroxide consumption during the 24-hr mVHP run was measured by continuously tracking the weight of the Vaprox<sup>®</sup> carboys at each vaporizer module. Total hydrogen peroxide consumption for all six vaporizer modules was 108,426 g (Table 17). The target hydrogen peroxide concentration level of 250 ppm was maintained throughout the duration of the run.

The injection rate at each vaporizer module was controlled by hydrogen peroxide concentration readings by the two monitors associated with each module. Due to the air leakage around the rear cargo door, noticed during the 24-hr VHP run, and the flow patterns generated within the cylindrical volume of the aircraft, the injection rate varied significantly among the vaporizer modules. Hydrogen peroxide consumption by the individual vaporizer modules for the 24-hr mVHP run is presented in Figure 38. As shown, vaporizer unit three gave an anomalous reading because a problematic data channel caused it to operate intermittently.

**Table 17: Consumption of H2O2 at Vaporizer Modules during 24-hr mVHP Run**

Vaporizer	H2O2 Consumed (g)
1	12495
2	21403
3	7043
4	20252
5	26432
6	20800
<b>Total</b>	<b>108426</b>

## 4. DISCUSSION

The C-141B decontamination demonstration is part of an ongoing congressionally funded partnership effort between ECBC and STE/STERIS Corporation, Inc. to develop and demonstrate mVHP technology for CBW agent decontamination. From this study, it can be determined that the VHP technology is a valid approach to the CBW decontamination of an aircraft interior. The main purpose of this effort was to determine whether VHP could be evenly distributed and its concentration sustained within a realistic environment for an effective period of time. The kill rate of the BIs and the recordings of the VHP sensors placed throughout the aircraft interior have conclusively proved that the VHP fumigant was effectively distributed. Furthermore, these tests also showed that a fluid dynamics model and the simple unidirectional placement of fans supported the VHP generator in effectively distributing the VHP within the complex geometries of the aircraft interior.



The *G. stearothermophilus* spore challenges to VHP/mVHP on board the C-141B aircraft were conducted in the form of commercial BIs and on a variety of aircraft related surfaces employing the same spore challenge. The difference between the two challenges was largely due to the contaminated surface material and to the 5.0% serum bio-burden level employed with lab prepared coupon surfaces.

Clearly the less difficult decontamination challenge is the commercial BI. The BI results presented in Table 8 are significant in that none of the *G. stearothermophilus* BIs showed any viable cells derived from spores treated during the 10.5- and 24-hr VHP runs, and 24-hr mVHP run. There were 3 positive BIs during the 5-hr VHP run (Table 8). Inspection of Figure 18 illustrates spore survival after 5 hr of VHP treatment across all three spore inoculated coupon surfaces, but complete VHP sporicidal efficacy after 10.5 hr of treatment.

The 24-hr mVHP exposure yielded what initially appeared to be an anomalous result (Figure 18). Upon recovery, a small number of the CARC coupons (three of six locations) and one glass coupon, showed spore growth. These coupons had been inoculated with *G. stearothermophilus* in 5.0% bovine serum. The use of bovine serum instead of an aqueous buffer in the spore preparation is the probable explanation for the appearance of the spore growth. The standard NATO acceptance for spore preparation is an aqueous buffer and not serum.

Previous laboratory efficacy studies have demonstrated that mVHP is less biocidal than VHP. Despite this feature, the 24-hr mVHP run, summarized as Figure 18 demonstrates reasonable efficacy. The data yielded one of three positive glass coupons taken from one of the six total locations sampled. Similarly, one of three CARC coupons at one of the six locations sampled was also positive. Interestingly, both positive coupons (out of 54 coupons) were sampled on the starboard side of the aircraft and therefore may have been influenced by distribution considerations.

Future studies to examine the impact of serum concentration levels on VHP/mVHP sporicidal efficacy are being planned. Two questions remain outstanding: serum level as bio-burden and the appropriateness of serum for use as bio-burden. The selection of serum as bio-burden originates from its use in medical instrumentation sterility testing where serum was used to represent a blood contaminant. In subsequent VHP/mVHP decontamination trials, bio-decontamination tests will be conducted with lower bio-burden.

The HD simulant, CEPS, test results demonstrated that only low concentrations were recovered from some of the test coupons following the 10- and 24-hr test runs. With the exception of one replicate, no CEPS was recovered from the 5-hr test coupons. The threshold workplace exposure limit (WEL) for HD is 0.003 mg/m<sup>3</sup>. Correlations performed in this study show that CEPS is slightly more persistent than HD, which, when taken with the oxidative conversion similarities of the two, suggests that the 24-hr samples were close to the Standardized NATO Agreement 4521 threshold concentration for HD WEL. The 5-hr test samples were well below this level. The performance of the 24-hr VHP run was less than optimal because of leaks in the aircraft tail.



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## GLOSSARY

APG	Aberdeen Proving Grounds
CRADA	Cooperative Research and Development Agreement
Ct	concentration time
CBW	chemical biological warfare
CW	chemical warfare
DoD	Department of Defense
ECBC	Edgewood Chemical Biological Center
GSA	General Services Administration
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
hr	hour or hours
IAW	in accordance with
kW	kilowatt
min	minutes
mVHP <sup>®</sup> , mVHP	reference to Steris' registered "modified vaporous hydrogen peroxide" procedure
PEL	permissible exposure limit
ppm	part-per-million
RH	relative humidity
STE	Strategic Technology Enterprises, Inc., a subsidiary of STERIS Corporation
VHP <sup>®</sup> , VHP	reference to Steris' registered "vaporous hydrogen peroxide" procedure